The clinical, radiographic, histological and ultrastructural results after Anterior Cruciate Ligament reconstruction using autografts

Michael Svensson

Department of Orthopaedics
Institute of Surgical Sciences, Sahlgrenska Academy at Göteborg University
Göteborg, Sweden
2008
ISBN 978-91-628-7318-9

The clinical, radiographic, histological and ultrastructural results after Anterior Cruciate Ligament reconstruction using autografts

Michael Svensson
Göteborg 2008

The principal aims of the study were to perform a long-term analysis of the patellar tendon structure after harvesting its central third as an autograft during ACL reconstruction. The patellar tendon underwent long-term serial morphological evaluations using MRI, histological evaluations at two and six years and ultrastructural evaluation six years after the harvesting procedure. Furthermore, the results after ACL reconstruction using BPTB or ST/G autografts were compared in a prospective study in a group of exclusively female patients.

All the patients underwent a standardised rehabilitation programme involving full range of motion exercises and full weight-bearing immediately after the reconstruction.

Nineteen patients underwent serial MRI examinations of the donor site six weeks, six months, two years and six years after the harvesting procedure. The study revealed that the patellar tendon had not normalised morphologically, compared with the contralateral side, up to six years after the harvesting procedure.

Seventeen patients underwent an ultrasonography-guided biopsy procedure of the central and lateral parts of the patellar tendon at the donor site, two and six years after the harvesting procedure. On both occasions, an increase in cellularity and vascularity and deterioration in fibre structure were found in the biopsy specimens from both the central and lateral parts of the patellar tendon at the donor site compared with normal control tendons.

Biopsy specimens from 13 patients obtained at six years were also evaluated using transmission electron microscopy. The extracellular matrix was more heterogeneous in the specimens from both the central and lateral parts of the patellar tendon, compared with normal control tendon. Moreover, significantly more small fibrils were found in both the central and lateral parts of the patellar tendon, compared with normal control tendons.

In a prospective study involving 63 female patients, donor-site morbidity in the form of knee-walking problems was significantly more common after using the BPTB autograft than after using the ST/G autograft. In terms of knee laxity and functional outcome, no significant differences were registered.

To summarise, the patellar tendon does not appear to regain normal morphology, histology and ultrastructure, up to six years after harvesting its central third. It appears that there is a long-standing effect on the entire tendon and not just the central part from where the graft was initially harvested.

Keywords: anterior cruciate ligament, surgery, radiology, biopsy, histology, ultrastructure.

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-IV)

I. Does the patellar tendon normalise after harvesting its central third?

A prospective long-term MRI-study

Svensson M, Kartus J, Ejerhed L, Lindahl S, Karlsson J.

Am J Sports Med. 2004; 32; 34-38.

II. Ultrastructural collagen fibril alterations in the patellar tendon 6 years after harvesting its central third.

Svensson M, Movin T, Rostgård-Christensen L, Blomén E, Hultenby K, Kartus J.

Am J Sports Med. 2007; 35; 301-306.

III. A long-term serial histological evaluation of the patellar tendon in humans after harvesting its central third.

Svensson M, Kartus J, Christensen LR, Movin T, Papadogiannakis N, Karlsson J.

Knee Surg Sports Traumatol Arthrosc. 2005; 13; 398-404.

IV. A prospective comparison of bone-patellar tendon-bone and hamstring grafts for anterior cruciate ligament reconstruction in female patients.

Svensson M, Sernert N, Ejerhed L, Karlsson J, Kartus J T.

Knee Surg Sports Traumatol Arthrosc. 2006; 14; 278-286.

Content

Abbreviations

AB/PAS	**A**lcian **B**lue/**P**eriodic **A**cid-**S**chiff
ACL	**A**nterior **C**ruciate **L**igament
BPTB	**B**one-**P**atellar **T**endon-**B**one
COMP	**C**artilage **O**ligomereic **M**atrix **P**rotein
ECM	**E**xtra **C**ellular **M**atrix
GAGs	**G**lycos**A**mino**G**lycan**s**
HE	**H**ematoxylin and **E**osin
HPF	**H**igh-**P**ower **F**ield
IKDC	**I**nternational **K**nee **D**ocumentation **C**ommittee
LOM	**L**oss **O**f **M**otion
MRI	**M**agnetic **R**esonance **I**maging
OA	**O**steo**A**rthritis
ROM	**R**ange **O**f **M**otion
RSA	**R**adio **S**tereometric **A**nalysis
SD	**S**tandard **D**eviation
ST	**S**emi**T**endinosus
ST/G	**S**emi**T**endinosus/**G**racilis
TEM	**T**ransmission **E**lectron **M**icroscopy
US	**U**ltra**S**onography

Introduction

Anterior cruciate ligament (ACL) injuries have become an increasing problem in both top-level and recreational sports not only in males but females as well (4, 12, 22, 28). Whether or not this is due to the increasing equality of opportunity between women and men, it is still a fact, at least in the western world, that nowadays many former male-dominated sports such as soccer, handball, floorball and downhill skiing are also a normal arena for females. This means that females have a panorama of injuries equal to that of males. When it comes to serious knee ligament injuries, females are even over-represented, with an incidence of ACL ruptures two to nine times higher than that of males in different studies (4, 6, 12, 22, 28, 33, 35, 36, 73, 82, 84, 99, 100). It therefore appears to be important to perform gender-specific analyses of the results after ACL reconstruction.

For decades, the patellar tendon was the most common autograft for an ACL reconstruction. The reconstruction was performed with an open or arthroscopic technique, using the central third of the patellar tendon with bone blocks at both ends. In many reports, this technique renders good and reproducible results (17, 23, 26, 81, 89). In global terms, the patellar tendon graft was the first choice until the last decade, when the use of the hamstring tendons, and first and foremost the semitendinosus tendon, started to increase. This has happened to some extent because of similar clinical results and less donor-site morbidity, as shown in several randomised, controlled studies (8, 17, 21, 26, 30, 32, 61, 76, 89). However, many surgeons still prefer the patellar tendon, especially in high-demand athletes (2, 5, 31, 67).

The optimal autograft should have qualities similar to those of the original ACL in terms of strength, stiffness, width and length. Furthermore, harvesting the optimal autograft should not cause additional problems for the patient, in the acute phase or in the long run (45). There are several reports in the literature relating to the drawbacks involved in using the patellar tendon as an autograft. Persistent clinical problems, especially in terms of donor-site morbidity, such as tenderness, anterior knee pain, patellar tendon shortening, knee extension deficit, disturbances in anterior knee sensitivity and inability to kneel and knee-walk are previously described (23, 51).

In the literature, there are reports that the patellar tendon does not normalise, as seen using Magnetic Resonance Imaging (MRI) assessments in the short or mid-term after harvesting its central third (18, 49, 62). No prospective studies have as yet been conducted to determine whether the patellar tendon recovers its normal radiographic appearance in the longer perspective. If the patellar tendon does not normalise radiographically, it is reasonable to believe that it will also experience histological changes. From animal studies in goat and dog models, it has been reported that, after harvesting its central third, the patellar tendon does not

normalise, at least in the short term. This has been seen histologically in the light microscope and ultrastructurally in the transmission electron microscope (TEM) (58, 83).

For ethical reasons, it is generally impossible to perform experimental tendon studies in live humans. However, ACL reconstruction using the patellar tendon autograft offers a unique opportunity, using a human model, to study the tendon response to surgical injury and the healing process.
The reports in the literature on the histological appearance of the patellar tendon in humans after harvesting its central third are sparse and contradictory. There are case reports describing an almost normal tendon, while others report abnormal tissue composition in both the central and peripheral parts (52, 75). Ultrastructural changes in fibril size distribution in tendons after spontaneous tendon ruptures have been reported (46, 64). However, no medium- or long-term studies of the ultrastructural appearance using the electron microscope in humans, after harvesting the central third of the patellar tendon, can be found in the literature.

The patellar tendon is still a very common autograft for ACL reconstruction. If it fails to normalise, even in the long term, after the harvesting procedure, this could affect the choice of a suitable graft both for primary ACL reconstruction and for revision ACL surgery. The corresponding consideration can be made for donor-site morbidity in terms of anterior knee pain and knee-walking ability. As a result, the histological and ultrastructural appearances of the patellar tendon in the long term are of specific interest, as this might have implications for future graft choice recommendations.

Review of the literature

Focus of the review

The main focus of this thesis is the behaviour of the patellar tendon after its central third has been harvested as a graft for ACL reconstruction. This has been investigated and assessed using three different methods, MRI, light microscope and electron microscope, in order to describe the tendon macroscopically, histologically and ultrastructurally. Moreover, clinical comparisons were made of the results after using bone-patellar tendon-bone (BPTB) and hamstring tendon autografts for ACL reconstruction in females.

MRI

Several imaging studies reveal that the patellar tendon at the donor site does not normalise in the short or medium-term after harvesting its central third. The thickness of the patellar tendon increases, at least up to two years post-operatively, irrespective of whether or not the defect is sutured (10, 11, 18, 53, 54, 62, 68, 75). Wiley and co-workers and Kartus and co-workers have made corresponding findings using ultrasonography (US) (52, 102). The only one of these studies indicating normalisation in the short term is the study by Meisterling and co-workers (68). In their study, the width and thickness of the patellar tendon were slightly increased, but not to any significantly different degree from the normal tendon, two years after the reconstruction.

There are no reports in the literature indicating that the patellar tendon normalises in the long term, as seen on MRI.

The finding that the patellar tendon does not normalise within two to three years, as seen on MRI, is not unique. The corresponding finding has been reported for the Achilles tendon after rupture (63, 69).

Light microscopy and histology

The reports in the literature on the histological appearance of the patellar tendon after harvesting its central third are sparse and contradictory. Nixon and co-workers reported that the histological appearance is indistinguishable from that of normal tendon in biopsies taken from two humans approximately two years after harvesting the central third of the patellar tendon (75). Kartus and co-workers reported abnormal tissue composition in both the central and peripheral parts of the patellar tendon in 19 humans after harvesting its central third. However, the follow-up period was only two years (52). Similar findings have been reported by Battlehner and co-workers (9). They obtained open biopsies from eight humans, a minimum of 24 months after ACL reconstruction using patellar tendon autografts. Using light and electron microscopy, they found that the patellar tendon did not regain the appearance of normal tendon during this period. However, in their study, the donor-site gap was closed surgically during the ACL reconstruction.

Using a goat model, Proctor and co-workers reported that the donor site, despite looking normal on MRI, revealed abnormal tissue composition when the biopsies were evaluated both histologically and ultrastructurally. They found ill-defined fascicles, woven collagen fibrils, poorly aligned with the longitudinal axis of the patellar ligament, in the central part of the tendon, 21 months after the harvesting procedure (83). Correspondingly, in a study of lambs using a light microscope, Sanchis-Alfonso and co-workers found that the regenerated tissue at the harvest-site defect did not have the histological appearance of normal patellar tendon (87). In a dog model, Burks and co-workers found that the entire patellar tendon was involved in scar formation three and six months after harvesting its central third (15).

Electron microscopy and ultrastructure

Studies of the patellar tendon in humans after harvesting its central third using an electron microscope are even more sparse than light-microscopic studies. The previously mentioned study by Battlehner and co-workers reported that the tendon does not recover “ad integrum” after a minimum of two years (9). Using the electron microscope in a dog model, LaPrade and co-workers reported that the reharvested central third from the loosely closed defect in the patellar tendon displayed increased fibril size and fibril packing at six months, compared with control tendons (58). However, at twelve months, no significant differences were registered. Using a goat model, Proctor and co-workers reported that the ultrastructure of the repair tissue, from the central third of the patellar tendon, was mainly composed of collagen fibrils with a small diameter. This was noted 21 months after harvesting the central six mm of the patellar tendon (83).

ACL injuries, graft choice and clinical results after reconstruction in females

The results after ACL reconstructions are not normally analysed separately for females and males. It is, however, quite possible that there could be gender-specific differences, especially in terms of laxity and donor-site morbidity.

Interest in evaluating and comparing the results after ACL reconstruction using either BPTB or hamstring (i.e. semitendinosus and/or gracilis, ST/G) autografts is well documented (5, 8, 17, 21, 23, 25, 26, 30, 60, 81, 89, 104).

For many years, the BPTB autograft was advocated as the gold standard (8, 23, 25, 32), but disadvantages such as donor-site morbidity, remaining patello-femoral pain and, in the worst case scenario, patellar tendon rupture or patellar fractures has led to an increasing interest in using ST/G autografts (8, 17, 26, 30, 32, 89).

Increased knee laxity over time after using ST/G autografts is one important post-operative difference to be reported when comparing the two methods (17, 27, 30, 32, 81, 103). However, in terms of objective and functional outcomes, the two methods appear to be similar in many studies (17, 23, 26, 32, 81, 89). Other disadvantages of the ST/G graft that have been discussed in the literature are

weakness in deep flexion and an increased risk of tunnel widening on the tibial side (2, 39, 74).

The incidence of an ACL rupture in females is two to nine times higher compared with males (6, 36, 99, 100). Possible explanations are a combination of hormonal influences, a narrow intercondylar notch, increased ligamentous laxity, less muscular strength and increased knee valgus, compared with males (36, 99). Both Östenberg and co-workers and Söderman and co-workers found that a general increase in joint laxity was a risk factor for traumatic injuries in the lower extremities in female soccer players (77, 93).

However, when analysing the post-operative outcome after an ACL reconstruction in terms of functional and objective assessments, no major gender differences have been found (6, 27, 99).

Aims of the study

- To perform long-term serial MRI assessments of the donor site in the same group of patients after harvesting the central third of the patellar tendon
- To evaluate and compare the long-term ultrastructural appearance of ultrasonography-guided patellar tendon biopsy specimens with those of normal control tendon
- To analyse the histological appearance of ultrasonography-guided biopsy specimens from the central and peripheral parts of the patellar tendon compared with normal control tendon, two and six years after the harvesting procedure
- To compare the outcome of ACL reconstruction after using the BPTB graft or four-strand ST/G graft in female patients

Patients

All the patients were diagnosed as having a unilateral ACL injury, clinically verified by a history of trauma, a positive Lachman test and/or positive pivot shift test or arthroscopic findings. The exclusion criteria were associated posterior cruciate ligament injury, more than +1 medial and/or lateral collateral ligament laxity, previous knee ligament surgery or known contralateral knee ligament injury and radiographically visible osteoarthritis (OA). All the patients that were included are presented in Table 1.

Table 1.

	Total number of patients initially included in Studies I-IV	**Comment**
Study I	19 patients	17/19 patients took part in the six-year assessment. The same patients comprised the initial study group in Studies I and III.
Study II	17 patients	13/17 patients had specimens which were evaluated. 17/19 of the patients included in Study III comprised the initial study group in Study II.
Study III	19 patients	17/19 patients took part in the six-year assessment. The same 19 patients comprised the initial study group in Studies I and III.
Study IV	63 patients	6/63 of the patients in Study IV were also among the 17 who underwent the examinations in Studies I-III.

NOTE: A total of 76 unique patients were included in Studies I-IV.

Study I

Nineteen consecutive patients (7 female and 12 male), who agreed to undergo serial MRI evaluations, were included in the study. Seventeen patients had an uninjured contralateral knee. One of the other two had previously undergone surgery to the contralateral side, involving an open reconstruction using the medial part of the patellar tendon as a graft, and the other had a conservatively treated ACL rupture on the contralateral side. The age of the patients at the index operation was a median of 27 (16-43) years and the operation was performed a median of 12 (2-192) months after the index injury. The post-operative assessments were performed a median of 25 (24-27) and 71 (68-73) months after the index operation.

Study II

Thirteen consecutive patients (6 female and 7 male), who had undergone ACL reconstruction using central-third patellar tendon autografts, were included in the study. The median age of the patients at the index operation was 27 (16-43) years and the operation was performed 12 (2-192) months after the index injury.

Study III

Seventeen consecutive patients (7 female and 10 male), who had previously undergone US-guided biopsies two years after the ACL reconstruction using patellar tendon autografts, were included in the study. The median age of the patients at the index operation was 27 (16-43) years and the operation was performed 12 (2-192) months after the index injury (52).

Study IV

Sixty-three consecutive female patients with a symptomatic unilateral ACL rupture, who were scheduled for ACL reconstruction using either an ipsilateral BPTB graft or an ipsilateral quadruple ST/G graft, were included in the study. One patient in each group underwent revision ACL surgery during the follow-up period. These two patients were excluded, leaving 61 patients. Follow-up was performed on 59/61 (97%) patients, as two patients were lost to follow-up. The comparisons between the treatment groups were based on 28 patients in the BPTB group and 31 in the ST/G group.

- The BPTB group consisted of 28 females. Their median age was 28 (16-50) years. The pre-injury Tegner activity level was 8 (1-9). The median time between the injury and index operation was 11 (1-252) months and the follow-up examination was performed a median of 26 (23-30) months after the reconstruction.
- The ST/G group consisted of 31 females. Their median age was 25 (13-53) years. The pre-injury Tegner activity level was 7 (2-10). The median time between the injury and index operation was 19 (2-276) months and the

follow-up examination was performed a median of 25 (23-31) months after the reconstruction.

The groups were comparable in terms of age, injured side, time between the injury and index operation and length of follow-up period, as well as the cause of injury (Table 2).

Table 2.
The cause of injury

	BPTB (n=28)	ST/G (n=31)	Significance
Contact sport	19 (68%)	18 (58%)	n.s. (p=0.44)
Non-contact sport	7 (25%)	9 (29%)	
ADL		2 (6.5%)	
Work		2 (6.5%)	
Other	2 (7%)		

Associated intra-articular injuries, such as meniscal ruptures, which were found or addressed at the index operation or during the follow-up period, were registered in 21/28 patients in the BPTB group and 28/31 in the ST/G group (p=0.12).

Methods

Blinded observers

In *Study I*, two physiotherapists performed the pre- and post-operative clinical assessments.
In *Study IV*, one physiotherapist, who was not involved in the rehabilitation, performed all the pre- and post-operative assessments. The physiotherapist was blinded to the aim of the study, but not to the type of surgical technique that was used.

The surgical procedure

All the patients underwent ACL reconstruction by one senior surgeon using a standardised endoscopic technique.

In *Studies I-IV*, the BPTB technique was used for all the patients apart from the ST/G group in *Study IV*. The arthroscopic transtibial technique and interference screw fixation were used during the index procedures (56). The central third of the patellar tendon was harvested through two 25-mm long vertical incisions, one over the apex of the patella and the other just above the tibial tubercle. The graft was tunnelled subcutaneously under the paratenon with the aim of protecting the infrapatellar nerve and its branches and leaving the major part of the paratenon intact, as described previously by Kartus and co-workers (47). The proximal bone block was sized to 9 mm and the distal bone block to 10 mm. The bone tunnels were prepared in a standard transtibial fashion. The femoral tunnel was placed at approximately 10.30 in the right knee and 01.30 in the left knee and the tibial tunnel was placed anterior to the normal posterior cruciate ligament in the ACL footprint. A 7 mm and a 9 mm Acufex® (Acufex, Microsurgical Inc., Mansfield, MA, USA) “silk” interference screw were used on the femoral and tibial side respectively (Fig. 1).

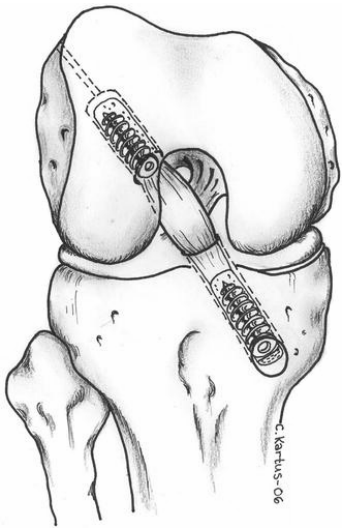


Figure 1.
Using the BPTB graft, a 7 mm and a 9 mm “silk” interference screw were used on the femoral and tibial sides, respectively. (Copyright Catarina Kartus)

In *Study IV*, in the ST/G group, the graft was harvested through an approximately 3-cm incision over the pes anserinus. The tendons were palpated and the sartorius fascia was incised parallel to the fibres of the fascia, just above the thicker and more distally inserted ST tendon. After the vinculae had been cut under visual control, both the ST and gracilis tendons were harvested with a semi-blunt, semicircular open tendon stripper (Acufex, Microsurgical Inc., Mansfield, MA,

USA). The tendons were prepared for a quadruple graft. Two no. 5 non-resorbable Ticron® (Sherwood Medical, St Louis, MO 63103, USA) sutures were used as lead sutures at the distal and proximal ends. Resorbable no. 1 Vicryl® (GmbH & Co. KG, D-22851 Norderstedt) sutures were used for the modified baseball stitches at the distal and proximal ends of the ST/G graft. The femoral tunnel was drilled through a medial portal and the tibial tunnel was drilled in a standard fashion. Both the femoral and tibial tunnels were placed at approximately the same locations as in the BPTB group. A 7 mm soft-threaded RCI® (Smith and Nephew, Inc, Andover, MA 01810, USA) interference screw was used on both the femoral and tibial sides (Fig. 2) (17).

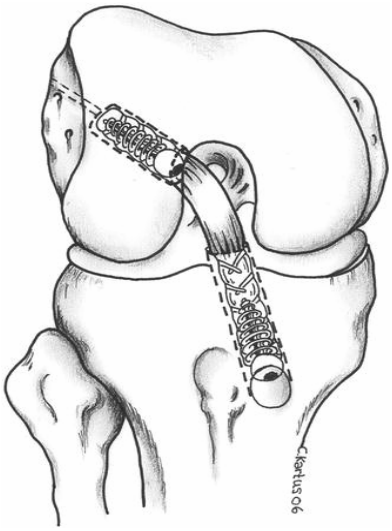


Figure 2.
Using the ST/G graft, a 7 mm soft threaded RCI® interference screw was used on both the femoral and tibial sides. (Copyright Catarina Kartus)

Registration of additional surgery

Additional surgery at the index operation was registered in the evaluation protocol (Fig. 9) and the patients' files.

MRI examination

One independent experienced radiologist evaluated all the MRI examinations. A Siemens™ (Erlangen, Germany) Magnetom 1.0 Tesla and a flexible knee coil were used. The knee was examined in slight flexion. A three-dimensional dual echo steady state (DESS) sequence was used and a three-dimensional reconstruction program was used to obtain axial reconstructions, from which values for the width and thickness were calculated through the mid-point along the length of the patellar tendon from the apex of the patella to the insertion at the tibial tubercle (Fig. 3). The mid-point of the patellar tendon at the donor site was then evaluated in terms of gap size (area corresponding to non-tendinous-like tissue signals), (Figs. 4 A-D) in the axial dimension. All the measurements were made using a Siemens™ evaluation unit using computerised distance measurements and standardised settings. The intraobserver SD of the difference between two measurements was 0.67 mm, as assessed by re-evaluating 10 randomly selected examinations of the thickness of normal patellar tendons, without knowledge of the primary result.

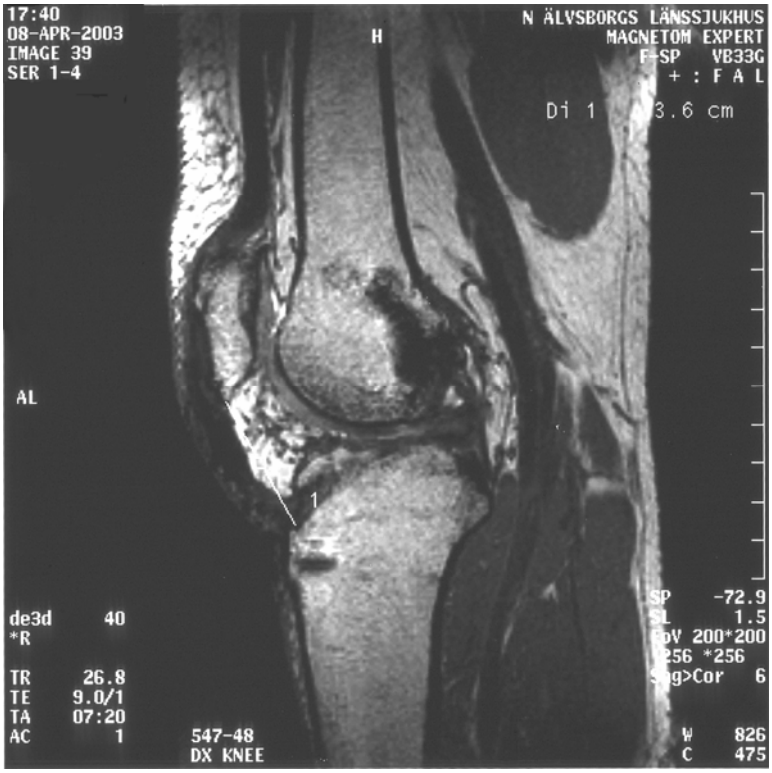


Figure 3.
Values of the width and thickness were calculated through the mid-point along the length of the patellar tendon from the apex of the patella to the insertion at the tibial tubercle. (Copyright Michael Svensson)

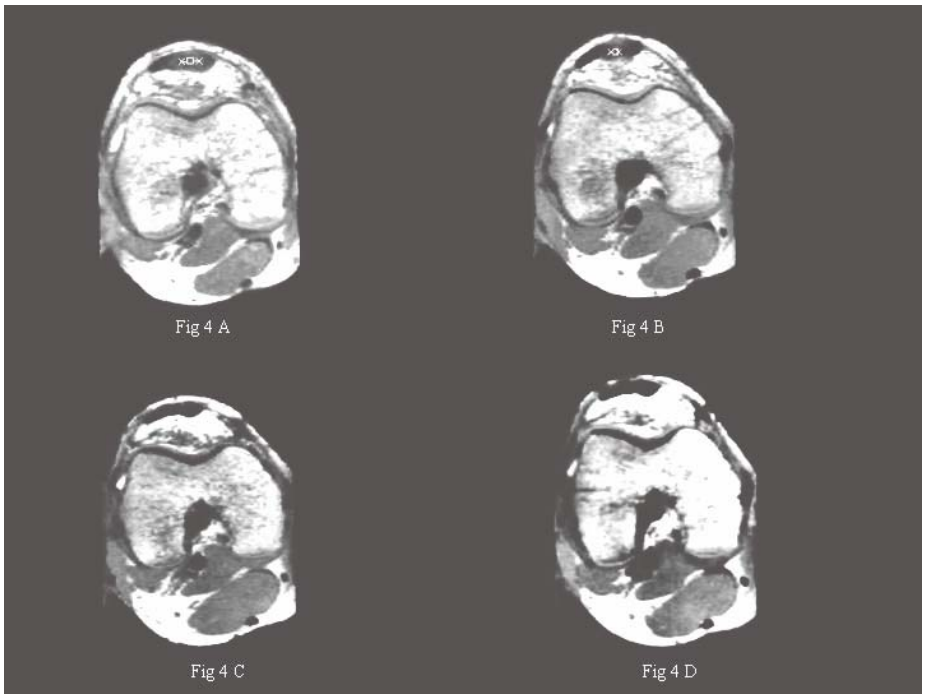


Figure 4 A-D.
The mid-point of the patellar tendon at the donor-site was evaluated in terms of gap size, as seen in this male patient who was 18 years old at the time of surgery. (Copyright Michael Svensson)

Biopsy procedures

On both biopsy occasions, four biopsy specimens (two central and two lateral) were obtained from each patient. The specimens were obtained under ultrasonography-guidance with a free-hand technique using a 1.2 mm Tru-cut Monopty™ instrument (Bard Inc., Covington, GA, USA) (Fig. 5). This is a lightweight metal handle with a pre-attached disposable biopsy needle. When fired, the gun needle moves in two steps. During the first step, the inner stylet punctures the target and, in the second step, an outer cannula follows the path of the stylet, covering the sample notch and thereby capturing the sample.

Local anaesthesia with adrenalin (5-10 ml) was given subcutaneously and in the fat pad of Hoffa. Through multiple small incisions, biopsy specimens were obtained from each patient, centrally from the donor-site gap area and peripherally from the lateral part of the patellar tendon. Each core biopsy specimen was placed separately in a coded tube. The samples which were obtained had a length of 5-10 mm and a maximum diameter of 1.2 mm.

This procedure has previously been shown to cause negligible discomfort and no short or long term complications for the patients (52).

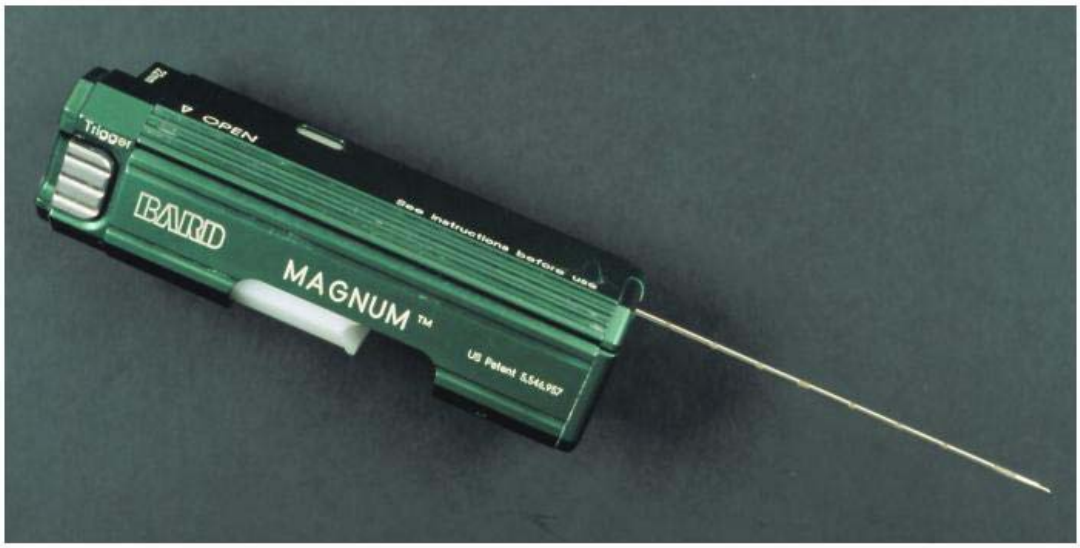


Figure 5.
The specimens were obtained under ultrasonography-guidance with a free-hand technique using a 1.2 mm Tru-cut Monopty™ instrument. (Copyright Michael Svensson)

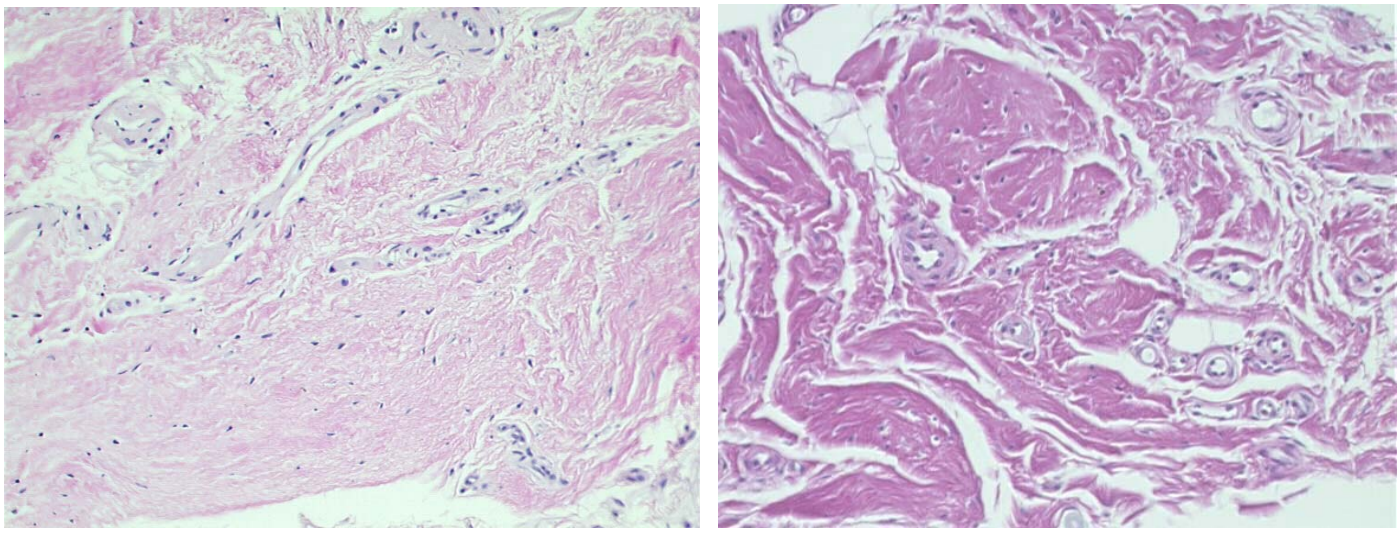
Control specimens

Tendon control specimens were obtained in an open fashion from the central third of the patellar tendon when harvesting the bone-patellar tendon-bone (BPTB) autograft in 11 other patients (one female and 10 males) treated with the same type of ACL reconstruction. The median age of the control patients was 27 (19-40) years. These patients had no previous history of pain in the patellar tendon region

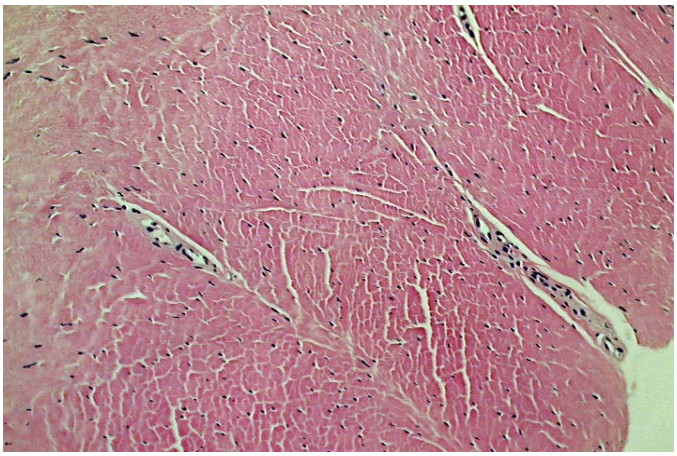
and, in previous arthroscopies, the anterolateral and anteromedial portals had been used.

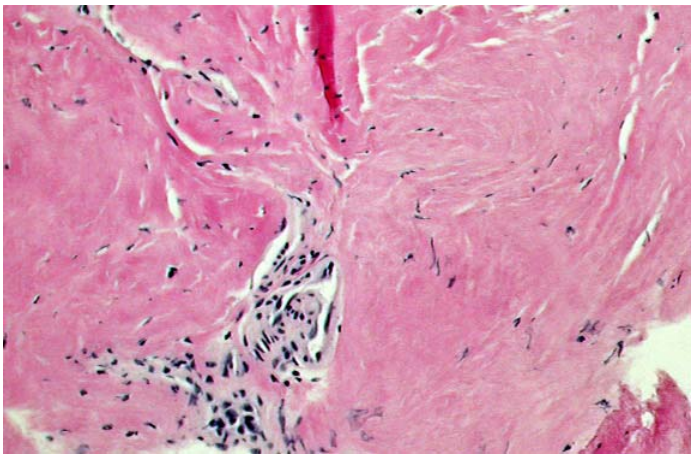
Histology

The biopsy specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned at 4-5μm. The sections were stained with hematoxylin and eosin (HE) to evaluate fibre structure, cellularity and vascularity (Figs 6 A and B, Figs 7 A and B and Fig. 8). The Alcian Blue (pH 2.5)/Periodic Acid-Schiff (AB/PAS) method was used to detect elevated levels of glycosaminoglycans (GAGs).



Figures 6 A and B.
Photomicrographs from the central repair tissue of the patellar tendon from a male patient who underwent ACL reconstruction at the age of 18 using the central third as an autograft. Specimen A was taken after two years and specimen B after six years. The fibre structure had deteriorated, the cellularity had increased and the vascularity had increased markedly on both occasions. (Approximate original magnification x200). (Copyright Springer Verlag)





Figures 7 A and B.
Photomicrographs from the lateral third of the patellar tendon from a male patient who underwent ACL reconstruction at the age of 26 using the central third as an autograft. Specimen A was taken after two years and specimen B after six years. The fibre structure had deteriorated moderately, the cellularity had increased slightly and the vascularity had increased on both occasions compared with normal controll tendon. (Approximate original magnification x200). (Copyright Springer Verlag)

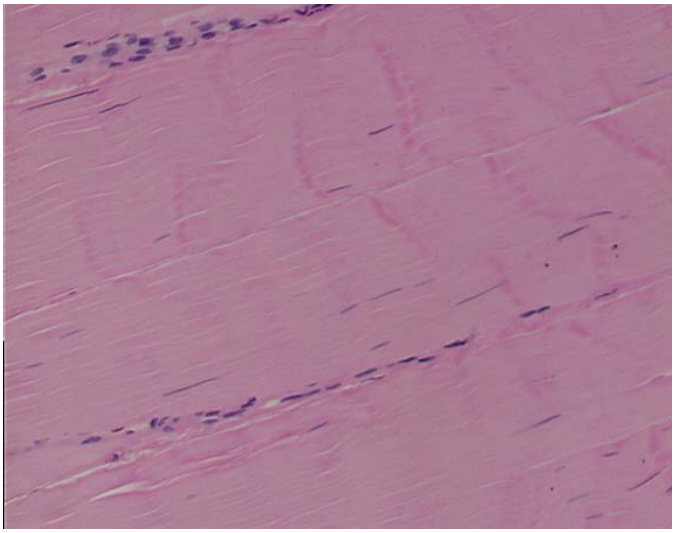


Figure 8.
Photomicrograph of a control specimen from the patellar tendon of a 25-year-old male patient who underwent ACL reconstruction using a central-third patellar tendon autograft. A small part of the tendon tissue was obtained for histological examination when the graft was trimmed. The specimen reveals normal tendon tissue with parallel tendon fibres, flat nuclei between dense fibres and small normal vessels. (Approximate original magnification x200). (Copyright Springer Verlag)

Evaluation of the biopsies

All the specimens were examined simultaneously using a light microscope by a pathologist and an orthopaedic surgeon, both with a specific interest in, and knowledge of tendon pathology. The biopsy specimens were evaluated using a semi-quantitative (non-parametric) grading system for the tendon pathology (52). Grading was based on a four-point scoring system (Table 3). The fibre structure, vascularity and level of GAGs were graded after examining the entire section. The number of cells was estimated in a high-power field (HPF) representative of the section. The biopsy specimens from the same patient were evaluated in paired fashion and the examiners knew which biopsy was taken at two years and six years, respectively.

In the case of the biopsy specimens taken at two years, the *mean* score for the two specimens obtained from the gap area was calculated for each of the four parameters and it was then used in all further analyses of the data. The same calculations were made for the two specimens obtained from the peripheral part of the patellar tendon. At six years, one biopsy specimen from each patient was evaluated from the central and peripheral parts of the tendon respectively. For every specimen and every parameter, agreement on the classification was reached by the two examiners.

Table 3.

Histological classification

A semi-quantitative four-point scoring system was used to evaluate the biopsies (52).

	Grade 0	Grade 1	Grade 2	Grade 3
Fibre structure	Straight parallel, packed fibres, with slight waviness	Slight separation of fibres, increased waviness	Separation of fibres, deterioration of fibres	Complete loss of fibre structure and hyalinisation
Cellularity	< 100 cells/high-power field (HPF)	100-199 cells/HPF	200-299 cells/HPF	> 300 cells/HPF
Vascularity	Vessels running parallel to the collagen fibre bundles in the septa	Slight increase in vessels, including transverse vessels in the tendon tissue	Moderate increase in vessels within the tendon tissue	Markedly increased vascularity with clusters of vessels
Glycosamino-glycans	No alcianophilia	Slight alcianophilia between the collagen fibres	Moderate increase in alcianophilia	Markedly increased alcianophilia forming blue lakes

Transmission electron microscopy (TEM)

Tendon specimens were collected and immediately fixed in 2% glutaraldehyde and 0.5% paraformaldehyde in 0.1M sodium cacodylate buffer containing 0.1M sucrose and 3mM $CaCl_2$ (pH 7.4) at room temperature for 30 minutes, followed by 24 hours at 4°C. The specimens were rinsed in 0.15 M sodium cacodylate buffer containing 3mM $CaCl_2$ (pH 7.4) and post-fixed in 2% osmium tetroxide in 0.07 M sodium cacodylate buffer containing 1.5 mM $CaCl_2$ (pH 7.4) at 4°C for two hours, then dehydrated in ethanol followed by acetone and embedded in LX-112 (Ladd, Burlington, Vermont, USA), for both longitudinal and transverse sectioning. Ultra-thin sections (approximately 40-50 nm) were cut and contrasted with uranyl acetate followed by lead citrate and examined in a Tecnai 10 microscope (Fei company, Eindhoven, the Netherlands) at 80 kV. Longitudinally oriented specimens were screened at low magnification (x3000) for morphological evaluation. From transversely oriented specimens, two randomly selected areas were taken and the fibril diameter was measured on printed copies (x101 000) using a Zeiss TGZ-3 particle-size analyser, grouped in five size classes (0-30 nm, 31-60 nm, 61-90 nm, 91-120 nm and >121 nm) and presented as the relative distribution (101). A minimum of 100 fibrils were analysed in each specimen.

The clinical examination test

A special protocol was developed for the pre-operative and/or post-operative clinical evaluations and was used in all studies (Fig. 9). The physiotherapists performed the evaluations, apart from the Lysholm knee-scoring scale, which was patient administered.

KNEE EVALUATION PROTOCOL, NÄL, TROLLHÄTTAN

Name _____ No _____

Sex ○ female ○ male

Age at op _____

Injuried side ○ left ○ right

Dominant side ○ left ○ right

Contralateral side normal ○ yes ○ no

Cause of injury ○ contact sport ○ none contact sport ○ ADL ○ work ○ other

Previous surgery ☐ none ☐ arthro men ☐ ACL ☐ diagn arthro ☐ open men ☐ other

Date of reconstruction _____

Type of operation ○ within 2 months after injury ○ more than 2 months after injury ○ Revision surgery
_____ weeks after injury
_____ months after injury
_____ months after recon

Injuries at reconstruction ☐ None but ACL ☐ LCL ☐ P cap ☐ Other ☐ Med men ☐ MCL ☐ Artrosis ☐ ACL ☐ Lat men ☐ PCL ☐ Cartilage

Type of graft ☐ BTB 1I ☐ BTB contra lat ☐ ST ☐ ST+G ☐ BTB 2I ☐ BTB reharvest ☐ FL ☐ Other

Index procedure ☐ ACL endo ☐ PCL ☐ LCL ☐ Men sut lat ☐ Part men lat ☐ ACL 2I ☐ MCL ☐ Men sut med ☐ Part men med ☐ Other

Twisted graft fixation ○ yes ○ no

Pretension ○ yes ○ no

Hospital stay _____ day/s

Brace ○ yes ○ no

Sickleave _____ days

Early problems (< 3 months) ☐ None ☐ Wound inf ☐ Deep inf ☐ Other ☐ Delayed wound healing ☐ Ext def ☐ Trombosis

Fp I ____ months pop Fp II ____ months pop Fp III ____ months pop FpIV ____ months pop

ROM	ext	flex	ext	flex	ext	flex	ext	flex	ext	flex
Preop	____	____	Fp I ____	____	Fp II ____	____	Fp III ____	____	Fp IV ____	____
contralateral side			ext ____	flex ____						

Manual MCL compared with contralateral side

Preop ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp I ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp II ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp III ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp IV ○ 0 ○ 1+ ○ 2+ ○ 3+

Manual LCL compared with contralateral side

Preop ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp I ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp II ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp III ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp IV ○ 0 ○ 1+ ○ 2+ ○ 3+

KT 1 000	15 lbs			20 lbs		
Prop	I	NI	D	I	NI	D
A						
P						
T						

KT 1 000	15 lbs			20 lbs		
Fp I	I	NI	D	I	NI	D
A						
P						
T						

KT 1 000	15 lbs			20 lbs		
Fp II	I	NI	D	I	NI	D
A						
P						
T						

KT 1 000	15 lbs			20 lbs		
Fp III	I	NI	D	I	NI	D
A						
P						
T						

KT 1 000	15 lbs			20 lbs		
Fp IV	I	NI	D	I	NI	D
A						
P						
T						

Manual Lachmann compared with contralateral side

Prop ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp I ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp II ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp III ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp IV ○ 0 ○ 1+ ○ 2+ ○ 3+

Total AP 70° compared with contralateral side

Prop ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp I ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp II ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp III ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp IV ○ 0 ○ 1+ ○ 2+ ○ 3+

Posterior sag 70° compared with contralateral side

Prop ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp I ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp II ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp III ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp IV ○ 0 ○ 1+ ○ 2+ ○ 3+

Pivot shift compared with contralateral side

Prop ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp I ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp II ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp III ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp IV ○ 0 ○ 1+ ○ 2+ ○ 3+

Reversed pivot shift compared with contralateral side

Prop ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp I ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp II ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp III ○ 0 ○ 1+ ○ 2+ ○ 3+ | Fp IV ○ 0 ○ 1+ ○ 2+ ○ 3+

Firm end point

Prop ○ yes ○ no | Fp I ○ yes ○ no | Fp II ○ yes ○ no | Fp III ○ yes ○ no | Fp IV ○ yes ○ no

Firm end point contralateral side ○ yes ○ no

One leg hop

Prop ____ % | Fp I ____ % | Fp II ____ % | Fp III ____ % | Fp IV ____ %

Parestesia (ant-post) x (prox-dist)

Prop ____ cm² | Fp I ____ cm² | Fp II ____ cm² | Fp III ____ cm² | Fp IV ____ cm²

Subjective femoro-patellar pain

Prop ○ yes ○ no | Fp I ○ yes ○ no | Fp II ○ yes ○ no | Fp III ○ yes ○ no | Fp IV ○ yes ○ no

Femoro-patellar score

Prop ____ | Fp I ____ | Fp II ____ | Fp III ____ | Fp IV ____

Donor site pain

Prop ☐ no ☐ yes ☐ apex ☐ mid ☐ tuberositas ☐ pes ☐ thigh dorsal ☐ screw | Fp I ☐ no ☐ yes ☐ apex ☐ mid ☐ tuberositas ☐ pes ☐ thigh dorsal ☐ screw | Fp II ☐ no ☐ yes ☐ apex ☐ mid ☐ tuberositas ☐ pes ☐ thigh dorsal ☐ screw | Fp III ☐ no ☐ yes ☐ apex ☐ mid ☐ tuberositas ☐ pes ☐ thigh dorsal ☐ screw | Fp IV ☐ no ☐ yes ☐ apex ☐ mid ☐ tuberositas ☐ pes ☐ thigh dorsal ☐ screw

Patellar tendon donor site union (palpatory)

Fp I ○ yes ○ no | Fp II ○ yes ○ no | Fp III ○ yes ○ no | Fp IV ○ yes ○ no

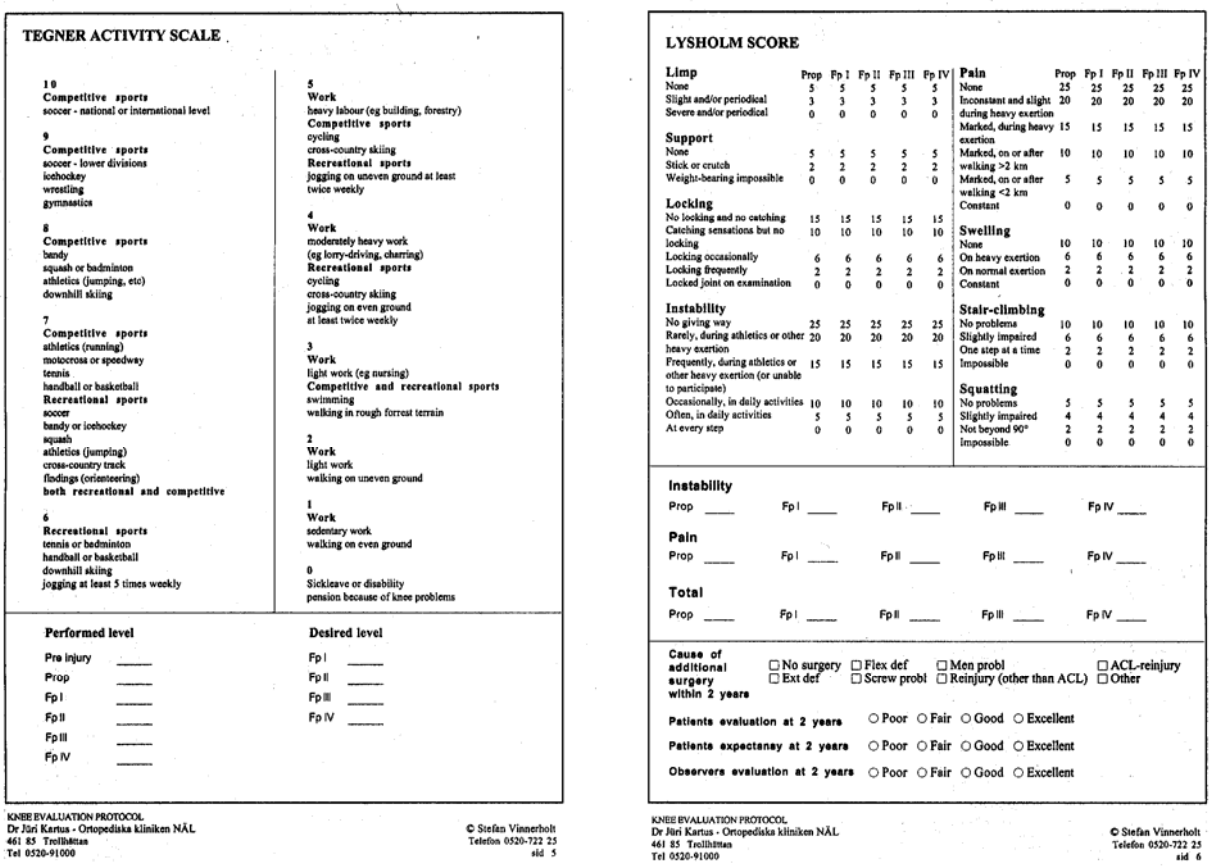
Kneeling

Preop ○ OK ○ Not pleasant ○ Difficult ○ Impossible | Fp I ○ OK ○ Not pleasant ○ Difficult ○ Impossible | Fp II ○ OK ○ Not pleasant ○ Difficult ○ Impossible | Fp III ○ OK ○ Not pleasant ○ Difficult ○ Impossible | Fp IV ○ OK ○ Not pleasant ○ Difficult ○ Impossible

Knee walking

Preop ○ OK ○ Not pleasant ○ Difficult ○ Impossible | Fp I ○ OK ○ Not pleasant ○ Difficult ○ Impossible | Fp II ○ OK ○ Not pleasant ○ Difficult ○ Impossible | Fp III ○ OK ○ Not pleasant ○ Difficult ○ Impossible | Fp IV ○ OK ○ Not pleasant ○ Difficult ○ Impossible

IKDC (Group grade)	Preop	Fp I	Fp II	Fp III	Fp IV
Subjective function	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D
Subjective symptoms	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D
Range of motion	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D
Ligament evaluation	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D
IKDC Final evaluation	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D	○ A ○ B ○ C ○ D



TEGNER ACTIVITY SCALE

10
Competitive sports
soccer - national or international level

9
Competitive sports
soccer - lower divisions
icehockey
wrestling
gymnastics

8
Competitive sports
bandy
squash or badminton
athletics (jumping, etc)
downhill skiing

7
Competitive sports
athletics (running)
motocross or speedway
tennis
handball or basketball
Recreational sports
soccer
bandy or icehockey
squash
athletics (jumping)
cross-country track
findings (orienteering)
both recreational and competitive

6
Recreational sports
tennis or badminton
handball or basketball
downhill skiing
jogging at least 5 times weekly

5
Work
heavy labour (eg building, forestry)
Competitive sports
cycling
cross-country skiing
Recreational sports
jogging on uneven ground at least twice weekly

4
Work
moderately heavy work
(eg lorry-driving, charring)
Recreational sports
cycling
cross-country skiing
jogging on even ground
at least twice weekly

3
Work
light work (eg nursing)
Competitive and recreational sports
swimming
walking in rough forrest terrain

2
Work
light work
walking on uneven ground

1
Work
sedentary work
walking on even ground

0
Sickleave or disability
pension because of knee problems

Performed level		Desired level	
Pre injury		Fp I	
Prop		Fp II	
Fp I		Fp III	
Fp II		Fp IV	
Fp III			
Fp IV			

KNEE EVALUATION PROTOCOL
Dr Jüri Kartus - Ortopediska kliniken NÄL
461 85 Trollhättan
Tel 0520-91000

© Stefan Vinnerholt
Telefon 0520-722 25
sid 5

LYSHOLM SCORE

Limp	Prop	Fp I	Fp II	Fp III	Fp IV
None	5	5	5	5	5
Slight and/or periodical	3	3	3	3	3
Severe and/or periodical	0	0	0	0	0
Support					
None	5	5	5	5	5
Stick or crutch	2	2	2	2	2
Weight-bearing impossible	0	0	0	0	0
Locking					
No locking and no catching	15	15	15	15	15
Catching sensations but no locking	10	10	10	10	10
Locking occasionally	6	6	6	6	6
Locking frequently	2	2	2	2	2
Locked joint on examination	0	0	0	0	0
Instability					
No giving way	25	25	25	25	25
Rarely, during athletics or other heavy exertion	20	20	20	20	20
Frequently, during athletics or other heavy exertion (or unable to participate)	15	15	15	15	15
Occasionally, in daily activities	10	10	10	10	10
Often, in daily activities	5	5	5	5	5
At every step	0	0	0	0	0

Pain	Prop	Fp I	Fp II	Fp III	Fp IV
None	25	25	25	25	25
Inconstant and slight during heavy exertion	20	20	20	20	20
Marked, during heavy exertion	15	15	15	15	15
Marked, on or after walking >2 km	10	10	10	10	10
Marked, on or after walking <2 km	5	5	5	5	5
Constant	0	0	0	0	0
Swelling					
None	10	10	10	10	10
On heavy exertion	6	6	6	6	6
On normal exertion	2	2	2	2	2
Constant	0	0	0	0	0
Stair-climbing					
No problems	10	10	10	10	10
Slightly impaired	6	6	6	6	6
One step at a time	2	2	2	2	2
Impossible	0	0	0	0	0
Squatting					
No problems	5	5	5	5	5
Slightly impaired	4	4	4	4	4
Not beyond 90°	2	2	2	2	2
Impossible	0	0	0	0	0

Instability
Prop ____ Fp I ____ Fp II ____ Fp III ____ Fp IV ____

Pain
Prop ____ Fp I ____ Fp II ____ Fp III ____ Fp IV ____

Total
Prop ____ Fp I ____ Fp II ____ Fp III ____ Fp IV ____

Cause of additional surgery within 2 years
☐ No surgery ☐ Flex def ☐ Men probl ☐ ACL-reinjury
☐ Ext def ☐ Screw probl ☐ Reinjury (other than ACL) ☐ Other

Patients evaluation at 2 years ○ Poor ○ Fair ○ Good ○ Excellent

Patients expectansy at 2 years ○ Poor ○ Fair ○ Good ○ Excellent

Observers evaluation at 2 years ○ Poor ○ Fair ○ Good ○ Excellent

KNEE EVALUATION PROTOCOL
Dr Jüri Kartus - Ortopediska kliniken NÄL
461 85 Trollhättan
Tel 0520-91000

© Stefan Vinnerholt
Telefon 0520-722 25
sid 6

Figure 9.
A special protocol was developed for the pre-operative and/or post-operative clinical evaluations and was used in all studies.

Specific evaluation tools

IKDC evaluation system

The IKDC classification was based on the patients' subjective evaluation of their knee function, such as symptoms and activity level, as well as knee laxity and range of motion (ROM) examinations, which were performed by the independent examiner. The results were graded as A (normal), B (nearly normal), C (abnormal) or D (severely abnormal). The worst qualification within the subgroup produced the subgroup qualification and the worst subgroup qualification produced the final evaluation as described by Hefti and co-workers (38). In the overall results, only the final IKDC classification was reported.

Lysholm knee-scoring scale

To avoid interviewer bias, the modified Lysholm knee-scoring scale was assessed by the patient using a self-administered questionnaire. The questionnaire did not show the scores for the alternative answers, as described by Höher and co-workers (41). It consists of eight items, where pain and instability each account for 25 of the total score of 100 points (95).

Tegner activity level
The Tegner activity level was assessed by the examiner during the course of the patient interview/examination. The score is graded between 0-10, where grades 0-4 cover activities of daily living and work and grades 5-10 indicate that the patient is able to participate in recreational or competitive sports (95).

Manual Lachman test
The manual Lachman test was estimated by the examiner as the amount of anterior drawer movement with the knee in 15°-20° of flexion. It was graded as 0, + (< 5 mm), ++ (5-10 mm) or +++ (> 10 mm), compared with the uninjured contralateral knee (55, 97).

Instrumented KT-1000 test
The examination was standardised by always using the same bench and always having the patients in the supine position. Both legs were placed on a thigh support with 30° of knee flexion (40). A footrest and a strap around the thighs kept the legs in a neutral position (29, 42). The arms were placed along the sides of the body and the patient was asked to relax (Fig. 10). The instrument was calibrated to zero before every displacement test. The anterior-posterior (A-P) displacement of the tibia in relation to the femur was registered at 20 pounds (89N). Firstly, the anterior displacement was registered and, subsequently, as the needle returned to zero, the posterior displacement was measured. The readings of the needle position were only accepted if the needle returned to zero ± 0.5 mm when the tension in the handle was released (19). In the literature a side-to-side difference of more than 3 mm in the anterior laxity measurement is defined as indicating an ACL injury (20, 66, 94).

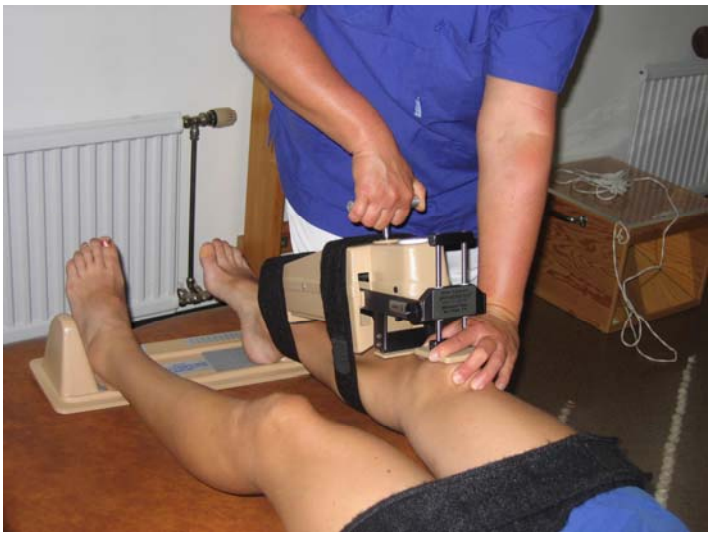


Figure 10.
The instrumented KT-1000 test. (Copyright Michael Svensson)

Range of motion (ROM)
The measurement was performed in the supine position using a hand-held goniometer graded in one-degree increments. The patients first made an active full extension followed by an active full flexion (14). The non-injured leg was always measured first and the side-to-side difference including hyperextension was calculated. If the measurements displayed a side-to-side difference of ≥ 5° in

either extension or flexion, the patients were categorically registered as having an extension and/or flexion deficit or not (72, 91). The examiner always made a visual check to ensure that the measured side-to-side difference appeared reasonable (14).

One-leg hop test

The one-leg hop test was performed by jumping and landing on the same foot with the hands behind the back (34). Three attempts were made for each leg and the longest hop was registered for each leg separately. A quotient (%) between the index and uninjured leg was calculated (37, 96), (Fig. 11). Side-to-side symmetry of at least 85% is recommended before returning to sporting activities (7, 20, 80).

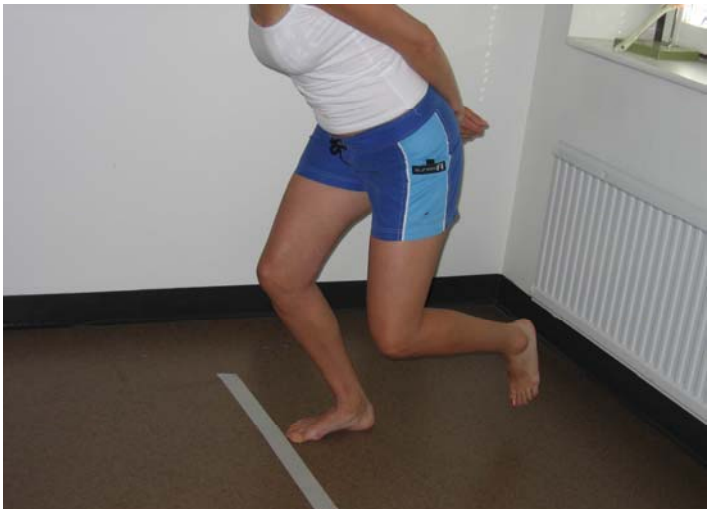


Figure 11.
The one-leg hop-test was performed by jumping and landing on the same foot with the hands behind the back. (Copyright Michael Svensson)

Knee-walking test

The knee-walking test was used in order to assess the discomfort compared with the contralateral knee. The knee-walking test was performed on the floor of the examination room at either the orthopaedic clinic or at the gym. The patients were not allowed to use any protection or clothing during the test. The patients knee-walked six steps forward and then subjectively graded the tests as OK (normal), unpleasant, difficult or impossible to perform, as described by Kartus and co-workers (50, 53), (Fig. 12).

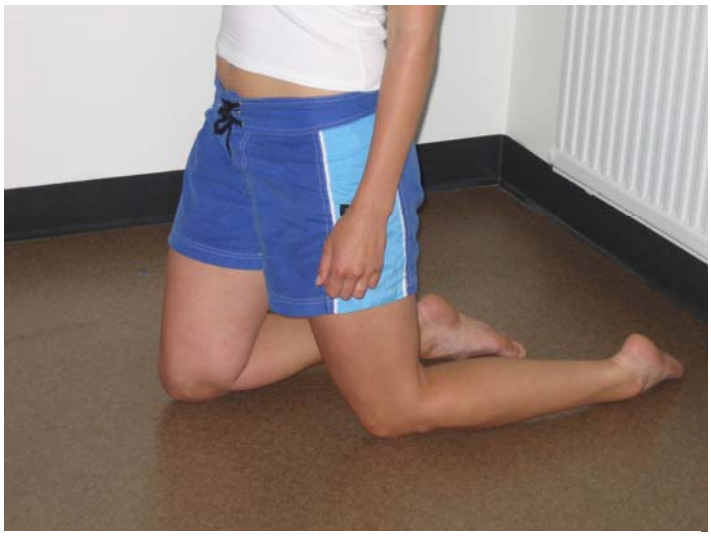


Figure 12.
The knee-walking test was used in order to assess the discomfort compared with the contralateral knee. (Copyright Michael Svensson)

Loss of skin sensitivity
The loss of or a disturbance in skin sensitivity was measured by the examiner palpating the anterior knee region. The length multiplied by the width was registered and the result is shown in cm^2 (50, 53), (Fig. 13).

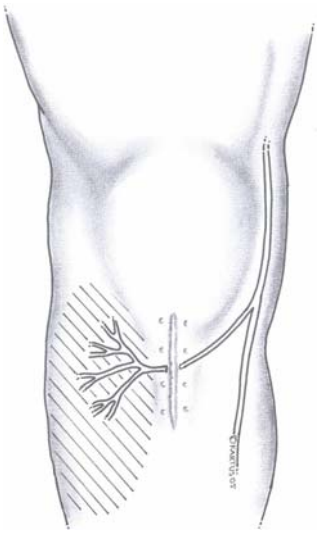


Figure 13.
The loss or disturbed skin sensitivity was measured by palpating the anterior knee region by the examiner. (Copyright Catarina Kartus)

Patients' subjective evaluation and expectation
The post-operative knee function was evaluated by the patient and graded as excellent, good, fair or poor. Correspondingly, the patients graded the extent to which the reconstruction had fulfilled their expectations.

Guidelines for the rehabilitation programme

Using our rehabilitation guidelines, the local physiotherapists created an individual training programme. Early weight-bearing was encouraged, as well as early full ROM training (44, 90). Closed kinetic chain exercises were started during the first post-operative week (57). Strength training between 30-0° with an external load was not permitted during the first six post-operative weeks. Running was permitted after three months and contact sports at the earliest after six months.

Statistical methods

Study I. For the MRI measurements, mean (SD) values are presented. Median (range) values are presented for all other measurements. Wilcoxon's rank sum test was used for the intra-individual comparisons between the longitudinal and trans-sectional observations in the cohort. The repeated measures analysis of variance and Scheffe's post-hoc tests were used for the longitudinal comparisons of the serial MRI assessments. Dichotomous variables were analysed using Fisher's exact test. A p-value of <0.05 was considered statistically significant.

Study II. The fibril size classes were first analysed using the χ^2 test for all three groups simultaneously. Because the χ^2 test revealed a significant difference in the size class distribution between the study groups, a subsequent analysis using the analysis of variance test was performed. A p-value of <0.05 was considered statistically significant.

Study III. Unless a mean value is indicated, the median (range) values are presented. Wilcoxon's rank sum test was used for the intra-individual comparisons between the longitudinal observations in the cohort. For comparisons between the patients and normal controls, the Mann-Whitney U test was used. A p-value of <0.05 was considered statistically significant.

Study IV. Median (range) values are presented. For comparisons of dichotomous variables between the groups, a χ^2 test was used. For both continuous and non-continuous variables, the Mann-Whitney U test was used. Wilcoxon's signed rank test was used for comparisons of the pre-operative and post-operative data within the groups. A p-value of <0.05 was considered statistically significant.

Ethics

All the studies included in this thesis were approved by the Human Ethics Committee at Göteborg University.

Summary of papers

Study I: *Does the patellar tendon normalise after harvesting its central third? A prospective long-term MRI study*

Introduction: The aim of this study was

- To perform long-term serial MRI assessments of the donor site in the same group of patients after harvesting the central third of the patellar tendon

The hypothesis was that the MRI examinations would reveal that the patellar tendon at the donor site appeared close to normal in terms of the thickness, width and appearance of the central part, six years after the index procedure.

Patients and Methods: Nineteen consecutive patients (7 female and 12 male), who agreed to undergo serial MRI evaluations, were included in the study. Seventeen patients had an uninjured contralateral knee (see page 14).

The clinical assessments involved the Tegner activity level, Lysholm knee-scoring scale, IKDC evaluation system, KT-1000 laxity measurements, manual Lachman test and one-leg hop test. The results of the laxity measurements and the one-leg hop test are only reported for those patients who had a normal contralateral ACL.

Results: At the six-month assessment, one patient was pregnant and therefore did not undergo the MRI assessment and, at the 71-month assessment, two patients were lost to follow-up. One had left the country and the other could not be located. During the period between the first and the second follow-ups, one patient ruptured his contralateral ACL.

On both follow-up occasions, the Tegner activity level, Lysholm knee-scoring scale, IKDC evaluation system, one-leg hop test and manual Lachman test revealed a significant improvement compared with the pre-operative values (Table 4).

The serial MRI examinations revealed that the size of the donor-site gap had decreased significantly (p=0.0001) between six weeks and six years after the harvesting procedure (Table 5). At two years, 3/19 patients displayed a donor-site gap with a tendinous-like tissue signal. The corresponding findings were made in 13/17 patients at six years (p=0.0006). A thinning of the central part of the patellar tendon compared with the surrounding tissue, measuring a mean of 2.2 (+/- 1.1) mm in width, was found in these 13 patients.

The thickness of the remaining patellar tendon at the donor site increased compared with the contralateral healthy side until two years after the index operation (p=0.003). However, the thickness (not including the thinned central part) decreased over time and had normalised compared with the contralateral side at six years (Table 5). The width of the patellar tendon at the donor site increased compared with the contralateral side, regardless of when the examination was performed (p=0.015), (Table 5).

Table 4.

Overall clinical results

A comparison of the pre-operative values and the values on both follow-up occasions using standard evaluation tools revealed a significant improvement in most parameters. The decrease in laxity as shown by the KT-1000, however, did not reach statistical significance. In terms of the one-leg hop test, KT-1000 and manual Lachman test, only patients with a healthy contralateral ACL were included in the statistical analyses. Median (range) values are presented.

	Pre-operative (total n=19)	**1st follow-up at 27 (24-29) months (total n=19)**	**2nd follow-up at 71 (68-73) months (total n=17)**	**Significance Pre-operative v 1st follow-up; 2nd follow-up**
Lysholm knee-scoring scale (points)	70 (52-85)	91 (65-99)	89 (50-100)	p=**0.0005;** p=**0.013**
Tegner activity level	3 (2-5)	7 (6-9)	6 (3-9)	p=**0.0001;** p=**0.0014**
IKDC normal/nearly normal	None	13/19 patients	10/17 patients	p=**0.0001;** p=**0.0001**
IKDC abnormal/severely abnormal	19/19 patients	6/19 patients	7/17 patients	As above
One-leg hop test (%) (n=17 pre-op and 27 months; n=14 at 71 months)	84 (0-107)	94 (68-132)	96 (71-120)	p=**0.0008;** p=**0.002**
KT-1000 total side-to-side difference (mm) (n=17 pre-op and 27 months; n=14 at 71 months)	4.5 (minus 2.5-8)	3 (minus 7-8.5)	3.5 (minus 2-9)	p=0.11; p=0.66
Manual Lachman (n=17 pre-op and 27 months; n=14 at 71 months)	0/+1/+2/+3 0/1/2/14	0/+1/+2/+3 9/8/0/0	0/+1/+2/+3 4/9/1/0	p=**0.0002;** p=**0.0008**

Significant values in bold

Table 5.
Serial MRI assessments
The gap and the thickness of the patellar tendon at the donor site decreased over time, but the width increased compared with the contralateral side, regardless of when the examination was performed. One patient had previously undergone a contralateral ACL reconstruction using a patellar tendon autograft and was thus excluded from comparisons requiring a normal contralateral side. Mean (±SD) values are presented.

	6 weeks (n=19)	**6 months (n=18)**	**27 months (n=19)**	**71 months (n=17)**	**Contralateral side (n=18)**	**Significance (serial analyses of the index side)**
Donor-site gap (mm, (±SD))	8.4 (±3.6)	4.7 (±2.7)	2.0 (±1.4)	0.5 (±0.9)	-	6 w v 6 mo; p=**0.0003** 6 mo v 27 mo; p=**0.01** 27 mo v 71 mo; n.s.
Thickness (mm, (±SD))	8.3 (±1.6)	7.2 (±1.1)	6.5 (±0.7)	4.8 (±1.0)	5.3 (±1.2)	6 w v 6 mo; p=**0.03** 6 mo v 27 mo; n.s. 27 mo v 71 mo; p=**0.001**
Width (mm, (±SD))	31.9 (±3.4)	30.2 (±3.6)	30.3 (±3.1)	30.3 (±3.3)	28.6 (±3.2)	6 w v 6 mo; n.s. 6 mo v 27 mo; n.s. 27 mo v 71 mo; n.s.

Significant values in bold

Conclusions: Six years after harvesting its central third and not closing the tendon defect, the patellar tendon still displayed radiographic abnormalities, as shown by the repeated serial MRI examinations.

Study II: *Long-term collagen fibril alterations in the patellar tendon after harvesting its central third*

Introduction: The aim of the present study was

- To obtain ultrasonography-guided biopsy specimens from the central and peripheral parts of the patellar tendon, approximately six years after the harvesting procedure, and to evaluate and compare the ultrastructural appearance of these specimens with that of normal control tendons

The hypothesis was that the patellar tendon would not regain a normal ultrastructural appearance in the long term.

Patients and Methods: Thirteen consecutive patients (6 female and 7 male), who had undergone ACL reconstruction using central-third patellar tendon autografts, were included in the study (see page 14). All the patients underwent ACL reconstruction by one senior surgeon using a standardised arthroscopic technique (see page 16). The biopsy procedure and the TEM procedure are described on pages 19 and 22.

Results: All the control specimens (n=10) were found to have a compact extracellular matrix (ECM) with regularly oriented collagen fibrils (Fig. 14 A). The cell density and shape varied, i.e. from flattened to swollen, indicating different activity status. Furthermore, no cell debris was found.

Specimens from the lateral parts (n=13) displayed a more heterogeneous ECM. In three of 13 specimens, the ECM was different compared with that of the controls (n.s; lateral v controls). In these specimens, collagen fibrils were oriented in different directions with more empty areas in between (Fig. 14 B). The cell density and shape varied, as in controls.

Specimens from the central parts (n=13) displayed an even more heterogeneous ECM. In this case, eight of 13 specimens were judged to have been influenced (p=0.003 central v controls). In these specimens, collagen fibrils were found to be randomly oriented, containing many empty areas (Fig. 14 C). Occasionally, areas containing cell debris were found. No differences in cell density and shape were noted.

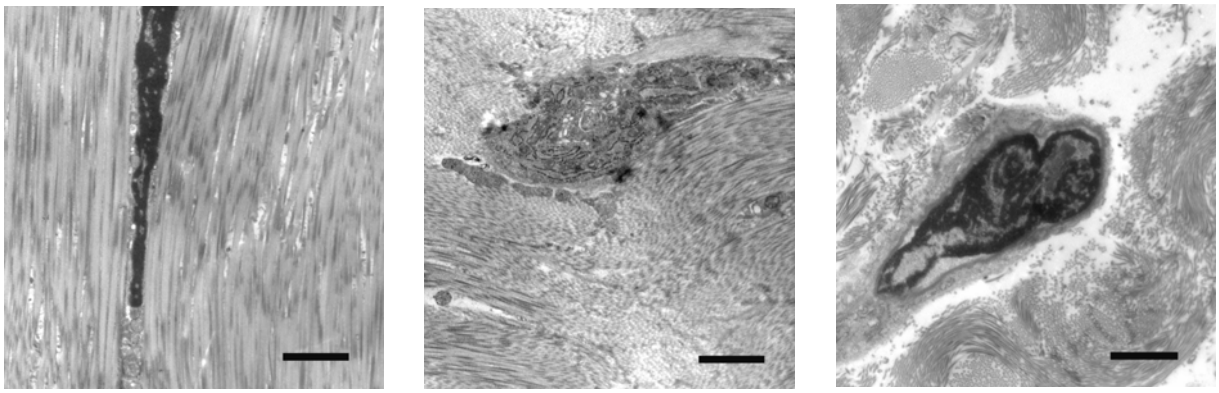


Figure 14 A-C.
Transmission electron micrographs from human control tendons (A), lateral parts (B) and central parts (C) of the index tendons in the study group (Bar = 2 µm; original magnification x3000). (Copyright Michael Svensson)

The relative distribution of the fibril diameter differed between the three groups ($p<0.001$). The fibril diameter in the control specimens displayed the most heterogeneous pattern and all the fibril size classes were present (Fig. 15 A and B). This was also found in the specimens from the lateral part of the patellar tendon. However, the two smaller fibril size classes (0-30 nm and 31-60 nm) were more dominant in the lateral specimens (88.4%) compared with the controls (72.5%) ($p<0.001$) (Fig. 15 C and D). In the central specimens, only the three smallest size classes were found and the fibril diameter between 31-60 nm was the most dominant (Fig. 15 E and F).

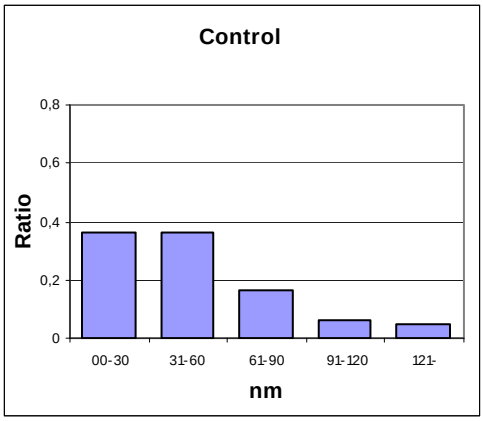


Figure 15 A

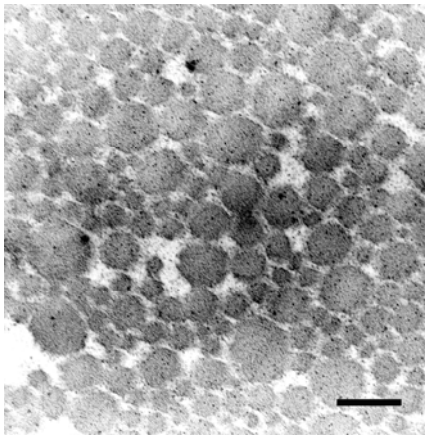


Figure 15 B

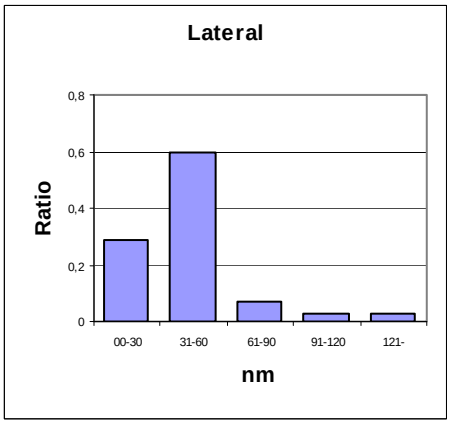


Figure 15 C

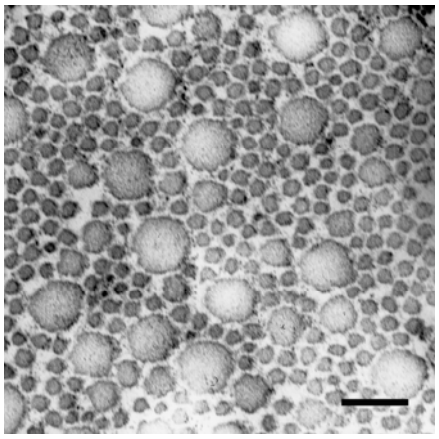


Figure 15 D

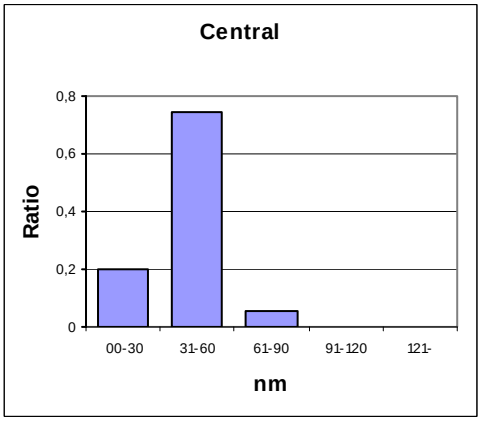


Figure 15 E

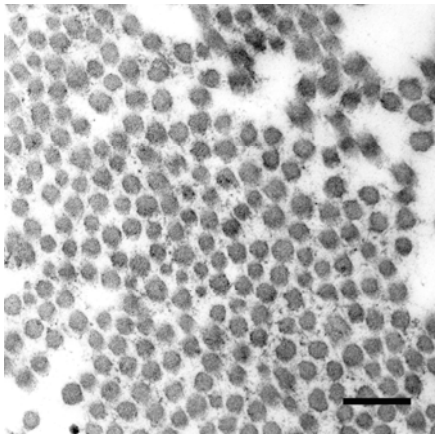


Figure 15 F

Conclusions: Six years after harvesting the central third of the patellar tendon, the tendon had not recovered a normal ultrastructure either in the central or in the peripheral part, as seen using TEM.

Study III: *A long-term serial histological evaluation of the patellar tendon in humans after harvesting its central third*

Introduction: The aim of the study was

- To obtain repeated US-guided biopsy specimens from the central and peripheral parts of the patellar tendon, two and six years after the harvesting procedure, and to evaluate and compare the histological appearance of these specimens. A further aim was to compare these specimens with specimens from normal control tendon.

The hypothesis was that, in the long term, the patellar tendon would not regain its normal histological appearance.

Patients and Methods: Seventeen consecutive patients (7 female and 10 male), who had previously undergone a US-guided biopsy procedure two years after ACL reconstruction using patellar tendon autografts, were included in the study (52) (see page 14). The surgical technique, biopsy procedure and the evaluation of the biopsy specimen are described on pages 16, 19 and 22.

Results:

All the individual ratings for fibre structure, cellularity and vascularity are presented in Table 6. Moreover, Table 7 summarises the data (median; range values) from the semi-quantitative histological four-point scoring system (0-3) for specimens from the central and peripheral parts of the patellar tendon at two and six years, as well as for the controls.

No major differences were seen between the two- and six-year biopsy specimens. In specimens from the central part of the tendon, the fibre structure had deteriorated slightly ($p=0.01$) and the cellularity had decreased ($p=0.02$) between two and six years after the harvesting procedure. Otherwise, no significant differences were found between the two- and six-year biopsy specimens (Table 7). On both occasions, the fibre structure had deteriorated significantly and the vascularity and cellularity had increased significantly compared with normal tendon. This was seen in both the central and peripheral parts of the tendon (Fig. 6 A and B and Fig. 7 A and B, Fig. 8, page 20-21), (Table 8). On both occasions, staining with the AB/PAS method was unable to detect increased levels of GAGs in either part of the tendon (Table 8).

Conclusions: Six years after the harvesting procedure, the patellar tendon still displayed an abnormal tissue composition with a persistent increase in cellularity and vascularity and fibre deterioration compared with the normal tendon. These histological changes were seen in both the central and the lateral parts of the tendon. We were able to verify the hypothesis that, in the long term, the patellar tendon would not regain its normal histological appearance after harvesting its central third.

Table 6.
Individual histological data

Patient	Fibre C2	Fibre C6	Cell C2	Cell C6	Vasc C2	Vasc C6	Fibre P2	Fibre P6	Cell P2	Cell P6	Vasc P2	Vasc P6
1	0.5	1	2	2	2	2	2.5	1	0	1	0.5	1
2	1	2	2	2	2	2	0	0	0	0	1	1
3	0.5	1	2	2	1.5	2	1	2	1	2	1.5	1
4	0.5	1	1	1	2.5	3	1.5	3	1	0	1.5	1
5	1	X	3	X	2	X	2	1	0	1	1	1
6	0	X	1	X	1.5	X	2	1	0	0	1	1
7	1	1	1	1	2	0	0	0	2	1	2	0
8	X	1	X	1	X	2	0.5	1	2	1	2	1
9	0	0	3	2	3	3	0	X	2	X	1.5	X
10	0.5	1	2	1	2.5	3	2	1	2	1	2	2
11	1	2	2	1	1.5	2	0	1	1	1	0	1
12	0	2	3	1	2.5	3	0	X	1	X	1.5	X
13	1	X	2	X	3	X	1	2	1	0	1.5	1
14	1	1	3	2	3	2	0	0	1	1	1	0
15	2	2	1	1	2	2	0.5	1	3	1	2	1
16	0.5	1	1	1	1.5	2	X	0	X	1	X	1
17	1	1	1	0	2	1	2	2	0	1	1	1

The histological semi-quantitative four-point scoring system (0-3) for three parameters: fibre structure (Fibre), cellularity (Cell) and vascularity (Vasc) in 17 patients. C2, C6, P2 and P6 indicate the central and peripheral parts at two and six years respectively. At two years for each patient, the mean value for two biopsies obtained from the same location in the patellar tendon is reported. At six years, only one biopsy from each part of the tendon was evaluated.

X indicates samples with an insufficient amount of tissue to be evaluated.

Table 7.
Summary of histological data

	Fibre 2 years	Fibre 6 years	Cell 2 years	Cell 6 years	Vasc 2 years	Vasc 6 years	GAGs 2 years	GAGs 6 years
Central part	0.75 (0-2)	1 (0-2)	2 (1-3)	1 (0-2)	2 (1-3)	2 (0-3)	0 (0-1)	0 (0-1)
Peripheral part	0.75 (0-2.5)	1 (0-3)	1 (0-3)	1 (0-2)	1.5 (0-2)	1 (0-2)	0 (0-1)	0 (0-1)

	Fibre		Cell		Vasc		GAGs
Controls	0 (0-0)		0 (0-0)		0 (0-0)		0 (0-0)

Summarised data (median; range values) from the histological semi-quantitative four-point scoring system (0-3) for fibre structure (Fibre), cellularity (Cell) and vascularity (Vasc) for specimens from the patellar tendon at two and six years respectively. There did not appear to be any major differences between the two- and six-year biopsies in either the central or the peripheral parts of the tendon. For comparison, data from 11 controls are shown. Abnormalities in the central and peripheral parts of the tendon compared with normal control tendon were found on both occasions.

Table 8.
Comparison between biopsies and normal control tissue

	Central 2 years	**Peripheral 2 years**	**Central 6 years**	**Peripheral 6 years**
Fibre structure	p=**0.0001**	p=**0.0017**	p<**0.0001**	p=**0.0004**
Cellularity	p<**0.0001**	p=**0.0007**	p<**0.0001**	p=**0.0003**
Vascularity	p<**0.0001**	p<**0.0001**	p<**0.0001**	p<**0.0001**

At both two and six years, the fibre structure had deteriorated significantly and the vascularity and cellularity had increased significantly compared with normal tendon. This was seen in both the central and peripheral parts of the tendon.

Study IV: *A prospective comparison of bone-patellar tendon-bone and hamstring grafts for anterior cruciate ligament reconstruction in female patients*

Introduction: The aim of the study was

- To compare the outcome of ACL reconstruction after using a BPTB graft or four-strand ST/G graft in female patients

The hypotheses were that, in the short term, the use of ST/G grafts for ACL reconstruction in female patients would cause less donor-site morbidity in terms of subjective anterior knee pain and would result in better knee-walking ability. Moreover, the use of ST/G grafts would reduce knee laxity and render good functional outcome to the same extent as the use of BPTB grafts.

Patients and Methods: Sixty-three consecutive female patients with a symptomatic unilateral ACL rupture, who were scheduled for ACL reconstruction using either an ipsilateral BPTB graft or an ipsilateral quadruple ST/G graft, were included in the study (see pages 14 and 16).

Results: The groups were comparable in terms of age, injured side, time between the injury and index operation and length of follow-up period (Table 9), as well as the cause of injury (Table 2, see page 15).

Table 9.
Preoperative data in the BPTB and ST/G groups

	BPTB	**ST/G**	**Significance**
Number of patients	28	31	
Age (years)	28 (16-50)	25 (13-53)	n.s. (0.28)
Injured side (right/left)	15/13	16/15	n.s. (0.88)
Preinjury Tegner activity scale	8 (1-9)	7 (2-10)	n.s. (0.92)
Time between the injury and index operation (months)	11 (1-252)	19 (2-276)	n.s. (0.23)
Follow-up period (months)	26 (23-30)	25 (23-31)	n.s. (0.77)
Associated injuries addressed or observed at the time of the index operation or during the follow-up period	21 (75%)	28 (90%)	n.s. (0.12)
Meniscal (medial and/or lateral)	12 (43%)	12 (39%)	
Meniscal and chondral	7 (25%)	15 (48%)	
Chondral		1 (3%)	
Other	2 (7%)		

At follow-up, there were no significant differences between the study groups in terms of the Lysholm knee-scoring scale or the Tegner activity level. Both groups improved

significantly between the pre-operative assessment and follow-up in terms of the Lysholm knee-scoring scale (BPTB; $p<0.01$ and ST/G; $p<0.001$) and the Tegner activity level ($p<0.01$ respectively). Both groups had a significantly lower Tegner activity level value at the follow-up compared with pre-injury ($p<0.001$), (Table 10). At follow-up, 64% (18/28) in the BPTB group and 45% (14/31) in the ST/G group had returned to a Tegner activity level of 6 or above ($p=0.35$).

There were no significant differences at follow-up between the study groups in terms of loss of motion (LOM), both extension and flexion (Table 10). The knee-walking ability was significantly better ($p=0.003$) in the ST/G group compared with the BPTB group at the two-year follow-up (Table 10). In the BPTB group, the knee-walking ability was significantly worse at follow-up than pre-operatively ($p=0.005$). The corresponding finding was not made in the ST/G group (Table 10).

The final IKDC classification at follow-up did not reveal any significant differences between the study groups ($p=0.92$), (Table 10).

Table 10.
The functional, objective and subjective results pre-operatively and at the two-year follow-up

		BPTB (n= 28)		ST/G (n=31)		Pre-operative BPTB v ST/G	Two-year follow-up BPTB v ST/G
		Pre-operative	**Two-year follow-up**	**Pre-operative**	**Two-year follow-up**		
Tegner activity level, median (range)		3 (1-9)	6 (1-9)*	4 (0-9)	5 (2-8)*	**0.01**	n.s. (0.35)
Lysholm knee-scoring scale (points), median (range)		65 (14-95)	87 (44-100)*	70 (28-99)	85 (51-100)**	n.s. (0.32)	n.s. (0.98)
One-leg hop test (%), median (range)		79 (0-110)	85 (53-132)*	74 (0-107)	85 (48-112)**	n.s. (0.41)	n.s. (0.87)
Missing values		2	1		1		
Extension deficit		11 (39%)	11 (39%)	11 (36%)	7 (23%)	n.s. (0.52)	n.s. (0.17)
Missing values		3 (11%)					
Flexion deficit		15 (54%)	13 (46%)	14 (45%)	20 (65%)	n.s. (0.27)	n.s. (0.16)
Missing values		3 (11%)	1 (4%)		1 (3%)		
Knee-walking test	**OK**	10 (36%)	7 (25%)*	19 (61%)	13 (42%)	n.s. (0.31)	**0.003**
	Not pleasant	13 (46%)	4 (14%)	6 (19%)	11(35/%)		
	Difficult	1 (4%)	2 (7%)	4 (13%)	5 (16%)		
	Impossible	3 (10%)	15 (54%)	2 (7%)	2 (7%)		
	Missing value	1 (4%)					
IKDC classification	**A (normal)**		3 (11%)		3 (10%)	n.s. (0.92)	
	B (nearly normal)		12 (43%)		13 (42%)		
	C (abnormal)		10 (35%)		14 (45%)		
	D (severely abnormal)		3 (11%)		1 (3%)		
Patients' evaluation	**Excellent**		6 (21%)		5 (16%)	n.s. (0.83)	
	Good		14 (51%)		17 (55%)		
	Fair		6 (21%)		8 (26%)		
	Poor		2 (7%)		1 (3%)		

Significant values in bold
* $p<0.01$, comparison between pre-operative and two-year follow-up values within the group
** $p<0.001$, comparison between pre-operative and two-year follow-up values within the group

The results of the knee-walking test were significantly poorer in the BPTB group compared with the ST/G group at the two-year follow-up.

The KT-1000 anterior and total side-to-side difference revealed no significant differences between the study groups either pre-operatively or at follow-up (Table 11). In both groups, the KT-1000 anterior and total side-to-side difference revealed a non-significant improvement between the pre-operative values and the values at the two-year follow-up. However, the manual Lachman test revealed a significant improvement in both groups (Table 11).

Table 11.
Laxity assessments according to the KT-1000 and manual Lachman tests pre-operatively and at follow-up in the BPTB and ST/G groups

		BPTB (n= 28)		ST/G (n=31)		Pre-operative BPTB v ST/G	Two-year follow-up BPTB v ST/G
		Pre-operative	Two-year follow-up	Pre-operative	Two-year follow-up		
KT-1000 anterior side-to-side difference		3.2 (-3.0-7.0)	2.0 (-6.0-10.0)	4.0 (-2.5-10)	3.5 (-3.0-9.0)	n.s (0.32)	n.s. (0.35)
KT-1000 total side-to-side difference		3.5 (-7-8.5)	2.8 (-7.0-10)	4.0 (-3.5-16.5)	3.0 (-2.5-8.5)	n.s. (0.76)	n.s. (0.83)
Missing value		4		1			
Manual Lachman test	**0**	0	26 (93%)*		26 (84%)*	n.s. (0.18)	n.s. (0.29)
	+1	2 (7%)	2 (7%)	2 (7%)	5 (16%)		
	+2	7 (25%)		14 (45%)			
	+3	19 (68%)		15 (48%)			

* p<0.001 between pre-operative and two-year follow-up values

The disturbance in anterior knee sensitivity at follow-up was a median of 54 cm^2 (0-324) in the BPTB group and 88 cm^2 (0-476) in the ST/G group (p=0.83) (Table 12). In both groups, there was no significant difference in terms of subjective anterior knee pain between the pre-operative and follow-up assessments (p=0.56 and p=0.32 respectively), (Table 12).

Table 12.
Disturbance of anterior knee sensitivity and subjective anterior knee pain pre-operatively and at follow-up in the BPTB and ST/G groups

	BPTB (n= 28)		ST/G (n=31)		Pre-operative BPTB v ST/G	Two-year follow-up BPTB v ST/G
	Pre-operative	Two-year follow-up	Pre-operative	Two-year follow-up		
Disturbance of anterior knee sensitivity (cm^2)		54 (0-324)		88 (0-476)		n.s. (0.83)
Missing values		2		4		
Subjective anterior knee pain	8 (29%)	11 (39%)	15 (48%)	11 (36%)	n.s. (0.10)	n.s (0.76)
Missing values		1 (4%)	1 (3%)	1 (3%)		

Conclusions: At the two-year follow-up, four-strand ST/G grafts produced results that were as good as those produced by BPTB grafts in terms of functional parameters and laxity. Donor-site problems presenting as knee-walking problems were significantly less common after using ST/G grafts. Furthermore, knee-walking problems were significantly more common in the BPTB group at the two-year follow-up than pre-operatively. The corresponding finding was not made in the ST/G group. We were thus able to verify the hypothesis.

Strengths and limitations of the studies

The strengths of *Study I* are its long follow-up period and the fact that it was performed on humans. To our knowledge, no other prospective long-term MRI studies of the donor site after harvesting its central third are found in the literature. One possible weakness in *Study I* is that there was no control group involved other than the contralateral healthy side. It would also have been interesting to have had one group of patients who underwent surgery using the medial or lateral third of the patellar tendon as a graft.

The strengths of *Studies II* and *III* are their long follow-up periods and the fact that they were performed on humans. Potential weaknesses are that no control groups were involved and no efforts were made to stain for nerve fibres and essential growth factors, such as e. g. Cartilage Oligomeric Matrix Protein (COMP).
One weakness that is shared by *Studies I-III* is that no biomechanical tests were performed. This means that it is not known for sure whether the quality, especially in terms of strength of the tissue in the central and peripheral parts of the tendon, was inferior compared with normal tendon.

The strengths of *Study IV* are its prospectively collected data and the fact that the same surgeon performed all the operations and the same blinded observer, who was not involved in the surgery or rehabilitation, performed all the pre- and post-operative evaluations. Apart from using a soft-threaded type of interference screw in the ST/G group and the fact that the femoral tunnel was drilled through the medial portal, the only variable that differed between the study groups was the type of graft. One potential weaknesses includes its non-randomised design.

Discussion

The use of patellar tendon autografts for ACL reconstruction is widespread and has been reported to render good and reproducible clinical results in several studies (16). It has even been called the golden standard, but is it “24 carat”? Interest in donor-site problems and the effects on the extensor mechanism, after removing one third of the patellar tendon, has increased. One important and as yet unanswered question is whether it is possible for the patellar tendon to compensate and adapt to the new biomechanical environment?

To perform in-depth evaluations, it was decided to use radiographic, histological and ultrastructural methods to analyse the patellar tendon after harvesting its central third.

MRI findings

The principal finding in *Study I* was that the patellar tendon, as seen using MRI examinations, did not regain a normal morphological appearance, up to six years after the harvesting procedure.

Three parameters were examined: the thickness, width and size of the central donor-site gap of the patellar tendon. We did not measure the length of the patellar tendon, after harvesting the BPTB graft with adjacent bone blocks from the patella and tibial tubercle, as there are obvious difficulties when it comes to finding reliable bony landmarks to measure between the proximal tibia and patella. RSA (Radio Stereometric Analysis) appears to be a more reliable method than MRI for this type of measurement. Adam and co-workers reported on ten consecutive patients, in whom the central third of the patellar tendon was used to reconstruct the ACL. A decrease in the length of the patellar tendon was observed in all cases, twelve months after the harvesting procedure. However, the shortening process only continued until the twelfth post-operative week, after which no further shortening occurred (1).

Our hypothesis in *Study I*, that the patellar tendon at the donor site would appear normal or close to normal, could not be verified. The thickness of the remaining patellar tendon increased compared with the healthy contralateral side until two years after the index operation. The thickness then decreased over time and had normalised at six years. The width increased, regardless of the time of examination. The donor-site gap decreased over time. However, six years after the harvesting procedure, a non-healed donor-site gap was still found in some of patients and a thinning of the central part of the patellar tendon was present in all patients (Table 5).

Several authors have reported that the patellar tendon does not normalise radiographically in the short term after harvesting its central third. However, to our knowledge, this is the first study of the same group of patients with serial MRI examinations up to six years after the harvesting procedure. Using MRI, Bernicker and co-workers reported findings similar to those described in *Study I* (11). In their

study, one year after harvesting the central third of the patellar tendon, the donor-site gap in the majority of the patients had not healed. Using MRI, Coupens and co-workers reported a significant increase in the cross-sectional area, mainly because of its increased thickness, up to 18 months after harvesting the central third of the tendon (18). Contrary to the findings in *Study I*, Meisterling and co-workers described near-normal width and thickness two years after harvesting the central third of the patellar tendon (68).

It therefore appears that *Study I*, as well as previous studies in the literature, indicates that a remodelling process continues for years after the harvesting procedure. It also appears that the "healing" and adaptation of the patellar tendon is a very slow process. The macroscopic appearance as seen using MRI had not normalised at six years, but these findings did not provide any information about the histological or ultrastructural factors which might explain this process. *Studies II* and *III* are therefore important contributions to further analyses of the course of the patellar tendon after harvesting its central third.

Histological and ultrastructural aspects

In a previous study, Kartus and co-workers examined biopsy specimens from the central and peripheral parts of the patellar tendon two years after the ACL reconstruction using central-third autografts (52). In that study, the histological examination demonstrated increased cellularity and vascularity and a deterioration in fibre structure compared with the control group. The histological changes occurred in the central and peripheral specimens of the tendon. No GAGs or collagen type III were found. This indicates that there are no similarities with the pathological tissue found in so-called tendinosis and that no collagen synthesis was present.

To our knowledge, no long-term histological evaluation in humans, from the same group of patients up to six years after the harvesting procedure, has previously been performed. *Study III* therefore offered a unique opportunity once again to examine biopsy specimens from the patients who were examined by Kartus and co-workers two years after the harvesting procedure (52).

In *Study III*, four parameters were examined: fibre structure, cellularity, vascularity and level of GAGs. The principal finding in *Study III* was that no major differences were seen between the two- and six-year biopsy specimens. In the specimens from the central part of the tendon, the fibre structure had deteriorated slightly and, in addition, the cellularity had decreased somewhat between two and six years after the harvesting procedure. Otherwise, no significant differences were found.
However, the histological situation was not static; a remodelling process appears still to be in progress six years after the harvesting procedure, however, slow. On both occasions, the fibre structure had deteriorated significantly and the vascularity and cellularity had increased significantly, compared with normal tendon specimens. This was seen in the specimens from both the central and peripheral parts of the tendon.

Staining for GAGs revealed no increase in either part of the tendon on any occasion. This means that the tissue in the patellar tendon did not display similarities with the findings in painful Achilles tendinosis or patellar tendinosis, as shown by Movin and co-workers (70).

The fact that the histological changes were found not only in the central specimens, where a surgical trauma had taken place, but also in the peripheral specimens which were not primarily affected by surgery is of particular interest. It appears that, by harvesting the central third of the patellar tendon, a histological situation is created which affects the entire tendon even at six years after surgery.

The principal finding in *Study II* was that, after harvesting its central third, the patellar tendon did not regain a normal ultrastructure as seen on biopsy specimens examined in TEM six years after the harvesting procedure. In the controls and in the biopsy specimens from the lateral part of the patellar tendon, the cell density and shape of the fibroblasts varied, i.e. from flattened to swollen, indicating different activity status. This was not seen in the biopsy specimens from the central part of the patellar tendon, indicating less cellular activity. Moreover, no modification towards a mature matrix with collagen fibrils of all sizes could be detected in biopsy specimens from the central part of the patellar tendon.
In specimens from both the central and the peripheral part of the patellar tendon, an irregularity in fibril structure and occasional cell debris were noted. The corresponding findings were not made in the control specimens.

One interesting finding was that specimens from the repair tissue in the central part of the patellar tendon consisted primarily of collagen fibrils with a small diameter. In specimens from the lateral part of the tendon, all subclasses of fibril size were detected, but an obvious displacement towards smaller fibril sizes was found.
Biomechanical testing of the patellar tendon would have been of great interest, but for ethical reasons it is not possible to perform these tests in humans. To our knowledge, no biomechanical testing of the patellar tendon in humans has been performed.
Using a goat model, Proctor and co-workers characterised the morphology, histology and ultrastructure of the patellar tendon twenty-one months after harvesting its central third. In their study, the similarities with the findings in *Studies II* and *III* in terms of increased cellularity and vascularity were obvious. Proctor and co-workers also found increased amounts of collagen fibrils with a small diameter in their ultrastructural examination. Their biomechanical testing revealed that the maximum force to rupture for the central part of the “healed” patellar tendon was reduced by 51% compared with normal tendon. Correspondingly, the ultimate stress to failure was reduced by 65% (83).
In a dog model, Burks and co-workers revealed a shortening of the tendon, three and six months after harvesting the central third of the patellar tendon. A scar process involving the whole of the patellar tendon and not only the central part was found.

When testing the biomechanical properties of the entire patellar tendon, they found a decreased load to failure and stiffness of 60% and 33% respectively in the previously harvested tendons compared with the controls (15).
Using a dog model, LaPrade and co-workers found an increased collagen fibril diameter in the reharvested central third of the patellar tendon six months after the index operation; however, by twelve months, no such differences were found. When biomechanical testing was performed, an increase in the stiffness and a decrease in the strain and load to failure of the regenerated central third of the patellar tendons was found (58).

Using both a light and an electron microscope, Battlehner and co-workers reported that the patellar tendon in humans does not recover "ad integrum" a minimum of two years after harvesting its central third and closing the tendon deficit (9).
Nixon and co-workers reported contradictory findings; the patellar tendon was indistinguishable from normal tendon using a light microscope two years after the harvesting procedure and leaving the defect open (75).

Taken together, many studies report persistent morphological, histological and ultrastructural changes, which are similar to the results in *Studies II* and *III*. The unique characteristics of *Studies II* and *III* include the fact that they were performed on humans and that the follow-up period was up to six years. Without exception, biomechanical studies in animal models reveal inferior tissue quality through a decrease in the maximum load to failure and ultimate stress to failure. Due to the similarities between the findings in *Studies II* and *III* and those in previous animal studies, it may be suspected that the tissue quality in the entire patellar tendon is inferior, probably on a permanent basis, after harvesting its central third, even in humans. However, *Studies II* and *III* did not actually show that the human patellar tendon was of inferior strength and quality six years after harvesting its central third.

A decrease in collagen fibril size can be seen with exercise and load and in repair tissue after harvesting the central third of the patellar tendon. A decrease in fibril diameter size in rats after mechanical stress shielding is described by Majima and co-workers and Muellner and co-workers (65, 71), while a decrease in fibril diameter due to the normal ageing of a tendon is reported by Tuite and co-workers (98). Josza and co-workers found mainly small fibril size classes in biopsy specimens taken from human tendons just after spontaneous rupture (46). Similarly, Magnusson and co-workers found a loss of larger fibrils at the Achilles tendon rupture site (64). Amiel and co-workers speculated that, in comparison with ligaments, tendons had less intrinsic adaptive potential at all ages and under all circumstances (3). All these reports and the findings in *Studies II* and *III* indicate that any changes in the tendon load will change the fibril size distribution.

It might be the case that, after skeletal maturity, the tendons lose the cellular adaptation capacity that is needed for modification of the tendon and then respond

poorly to all forms of biomechanical change. Adequate levels of COMP as well as probably also growth factors may be necessary for the formation of structurally competent collagen matrix, as shown by Smith and co-workers using a horse model (92). If these factors are missing, a degeneration of the tendon matrix might instead occur.

One interesting explanation has been presented by Lavagnino and co-workers, who reported that "isolated fibrillar damage in tendon fascicles resulted in an upregulation of interstitial collagenas mRNA expression and protein synthesis" (59). Moreover, this process may weaken the tendon and put more of the extracellular matrix at risk of further damage with subsequent loading. It is possible that the isolated collagen fibril damage has more of an impact on tendon health in the long term by altering the interaction between the cell and extracellular matrix rather than as a result of any initial structural weakness in the tendon itself, as suggested by Lavagnino and co-workers. This isolated fibrillar damage and reaction could therefore possibly partly explain the changes in the remaining two-thirds of the patellar tendon. The increased load on the remaining two-thirds of the patellar tendon, due to the inferior loading capacity of the central part, may cause isolated collagen fibril damage in the remaining part of the tendon (59).

In our opinion, one-third of the patellar tendon cannot be sacrificed without complications in terms of reduced biomechanical properties. At least six years after the harvesting procedure, the tendon does not recover normal morphology, histology and ultrastructural appearance and in all probability it also has reduced biomechanical properties. A greater understanding of the way changes in the tendon affect the extensor mechanism and donor-site problems, as well as the knee-joint kinematics, would be of interest. It is also our opinion that the findings presented in *Studies II* and *III* might be of functional importance for the knee joint. From a clinical point of view, it is possible that it might be easier to accept a loss of a structure completely, e.g. the hamstring tendons, rather than adapting to a new biomechanical environment after harvesting the central third of the adult patellar tendon.

If revision ACL surgery is needed, we recommend that graft alternatives other than reharvested patellar tendon should be considered. It is probable that the reharvested central third of the patellar tendon will have inferior biomechanical properties for at least six years after the harvesting procedure. If reharvested patellar tendon is used, the way a second surgical trauma would affect the parameters described in *Studies II* and *III* must be taken into consideration. The long-term histological and ultrastructural effects of a second surgical trauma to the patellar tendon are as yet unknown.

Gender perspectives with special reference to graft choice and clinical outcome

In *Study I*, the clinical results as evaluated using the Lysholm knee-scoring scale, Tegner activity level, manual Lachman test and the IKDC evaluation system improved significantly from the pre-operative evaluation to both the two- and six-year follow-up. Since *Study I* only involved a limited number of patients, operated on using the BPTB autograft, further clinical and gender-specific analyses and conclusions are not justified.

At follow-up, the most important, clinically significant difference between the two groups in *Study IV* was that the ST/G groups had a better ability to knee-walk. This is in line with previous studies (21, 78). In a study by Ejerhed and co-workers, this variable revealed the only significant difference between BPTB and hamstring tendon autografts (21). This knee-walking test has been used for evaluating anterior knee problems after ACL reconstruction in several publications (21, 48, 50, 53, 88). The ability to kneel on the floor and/or knee-walk is of importance in several occupations such as cleaning, firefighting and child care, as well as during leisure-time activities. Similar results, defined as kneeling and squatting, are discussed in other prospective studies comparing BPTB and ST/G autografts (5, 17, 23, 81). Feller and co-workers found that the BPTB group had a significantly increased prevalence of pain in kneeling and anterior knee pain, compared with the ST/G group, but there were no significant differences between the groups in terms of the severity of pain as measured using a Visual Analogue Scale (26).

One important finding in *Study IV* was that there were no significant differences between the study groups, in terms of knee laxity as measured with the KT-1000 arthrometer at the final follow-up examination. Freedman and co-workers performed a meta-analysis comparing patellar tendon and hamstring tendon autografts. They found 735 publications in which 21 patellar tendon studies and 13 hamstring studies were included. They found a significantly higher percentage of patients in the BPTB group with a side-to-side difference of less than 3 mm using the KT-1000 arthrometer test (30). However, when a four-strand semitendinosus and gracilis graft was used, as in *Study IV*, no differences in terms of knee laxity were found between the BPTB and ST/G grafts in several previous publications (5, 23, 89). Corry and co-workers found a significant increase in knee laxity in the BPTB group compared with the ST/G group (17). Furthermore, the females in the ST/G group had a significant increase in knee laxity compared with the males in the same group, as well as both males and females in the BPTB group (17). Gobbi and co-workers found no significant differences between the BPTB and ST/G groups, but the females in the ST/G group had a small increase in knee laxity (32). Comparing males and females after ACL reconstruction using ST/G autografts, Noojin and co-workers found no significant differences in the KT-1000 arthrometer measurements (76). In a prospective study comparing the results of ACL reconstruction using BPTB and ST/G, Pinczewski and co-workers found a significant increase in knee laxity in females compared with

males at the two-year follow-up, but not at the three- and five-year follow-ups. However, the females in the ST/G group had a significant increase in laxity compared with the females in the BPTB group at the two-year follow-up (81).
Ferrari and co-workers found a significant difference between males and females in the overall KT-1000 results, but no differences in the percentage of patients who had a side-to-side difference of more than 5 mm (27).

Females have been found to have increased knee laxity in normal knees compared with males, but this does not explain the difference in knee laxity after using the BPTB and ST/G grafts (13, 43, 85, 86). The only prospective study that was found apart from S*tudy IV*, comparing BPTB and ST/G autografts exclusively in females, did not reveal any significant differences in knee laxity between the groups (8). In *Study IV,* knee laxity as measured using the KT-1000 arthrometer was less at the follow-up than pre-operatively, but the difference was not statistically significant. One possible explanation might be the wide range of values both pre-operatively and at the follow-up examination, as well as the relatively small number of patients in the study groups. However, the manual Lachman test revealed a significant improvement in both groups.

In S*tudy IV*, no significant differences were found between the groups in terms of the Lysholm knee-scoring scale, Tegner activity scale level or IKDC final classification at the follow-up evaluation, which is in line with several other studies (17, 21, 23, 32, 60, 89). Fifty-four per cent of the BPTB group and 52% of ST/G group were classified as IKDC normal/nearly normal. This is lower compared with several other studies (17, 32, 81). However, similar results were reported by Eriksson and co-workers (23) in their prospective, randomised trial.

In S*tudy IV*, the Tegner activity scale was significantly higher at follow-up compared with the pre-operative value, but neither group returned to the same activity level as before the injury. The same finding was made in a prospective long-term follow-up study by Brandsson and co-workers (13). Contrary to males, females did not return to their pre-injury activity level to the same extent in the study by Noojin and co-workers (76). At the three-year follow-up, Feller and co-workers reported that 54% in the BPTB group and 52% in the ST/G group had returned to their pre-injury level of activity (26). This is in line with Wiger and co-workers, who reported that only 39% of the males and 40% of the females returned to their pre-injury activity level (99). According to Söderman and co-workers, 80% of the female soccer players gave up soccer due to symptoms from their ACL-injured knee (93). If females return to their pre-injury activity level to a lower extent than males, this is possibly caused by factors such as childbirth and other natural changes of interest in life rather than a truly inferior result.

The use of BPTB autografts may cause increased patellofemoral pain (23, 76, 89) and this could also explain the loss of motion (44, 50, 90). In *Study IV*, no significant

differences were found in terms of ROM or patellofemoral pain, which is in line with previous studies (21, 60). Feller and co-workers found a persistent extension deficit in the BPTB group, compared with the ST/G group, while Pinczewski and co-workers found that an increased percentage of the BPTB group had an extension deficit at the two-year follow-up but not at the five-year follow-up (26, 81). In the study by Shaieb and co-workers, the overall loss of motion was significantly higher in the BPTB group compared with the ST/G group and only one patient in the ST/G group registered an extension deficit (89).

It has been shown that knee-walking ability has an inverse correlation to the loss or disturbance of anterior knee sensitivity after ACL reconstruction using BPTB autografts (50). In *Study IV*, there were no significant differences in the loss of sensitivity between the study groups, which indicates that both graft-harvesting techniques may potentially jeopardise the infra-patellar branches of the saphenous nerve to a similar extent. Due to the location of the skin incisions in the present study, possible subcutaneous neuroma formations will bear weight during knee-walking after BPTB graft harvest but not after ST/G graft harvest. This theory has previously been proposed by Ejerhed and co-workers as one of the reasons for the difference in knee-walking ability found in *Study IV* (21). One important finding in *Study IV* was that the knee-walking ability was significantly worse at follow-up than pre-operatively in the BPTB group. This finding speaks in favour of using the ST/G graft in young female as well as male patients who are often involved in kneeling and knee-walking activities, especially during child-care activities.

Conclusions

- The patellar tendon still displayed an abnormal morphological appearance, as seen on MRI, six years after harvesting its central third as an autograft for ACL reconstruction.

- The patellar tendon, as examined using MRI, revealed an ongoing remodelling process between two and six years after the harvesting procedure.

- Histologically, the patellar tendon, when examined in the light microscope, revealed increased cellularity and vascularity and a deterioration in fibre structure six years after the harvesting procedure.

- The histological changes were seen not only in the central part of the tendon, where surgical trauma had taken place, but also in the peripheral part of the tendon.

- The ultrastructure of the patellar tendon, examined using TEM, revealed a deterioration in the fibres and the ECM in the central and peripheral part of the tendon six years after the harvesting procedure.

- The repair tissue in the central part of the previously harvested patellar tendon consisted primarily of collagen fibrils with a small diameter.

- In the lateral part of the tendon, all fibril diameter sizes were represented, although smaller size classes were significantly more common than in the control biopsy specimens.

- At the two-year follow-up, four-strand ST/G autografts produced results that were as good as those produced by BPTB autografts in terms of functional outcome and laxity, in women.

- Donor-site problems presenting in the form of knee-walking problems were significantly less common after using ST/G autografts compared with BPTB autografts at the two-year follow-up, in women.

- Knee-walking problems were significantly more common in the BPTB group at the two-year follow-up than pre-operatively.

Clinical relevance

The finding that radiographic, histological and ultrastructural evaluations of the patellar tendon in both the short and long term after harvesting its central third revealed abnormalities indicating that the tendon will probably never normalise. These findings also indirectly indicate that the quality of the tendon tissue is inferior compared with that of normal tendon. Reharvesting the central third of the patellar tendon for use as a graft in the event of ACL revision surgery being necessary can therefore not be recommended.

This study also showed that ACL reconstruction using ST/G autografts caused less donor-site morbidity in female patients in terms of knee-walking problems and could therefore be recommended. Moreover, ST/G autografts produced equal results in terms of function and laxity compared with BPTB autografts at the two-year follow-up.

Final considerations and the future

In this thesis, the findings and problems that can occur in patients who require reconstructive surgery after an ACL injury are discussed. The donor site at the patellar tendon has been thoroughly examined clinically, radiographically and histologically and by examining its ultrastructure. All the different evaluation methods indicate that the patellar tendon does not normalise after harvesting its central third. The findings in the study can be used as a model for what happens in both the short and long term when a tendon is subjected to a surgical trauma.

In recent years, the “gold standard” for ACL reconstruction, the BPTB autograft, has gradually been replaced by hamstring tendon autografts. The change has taken place with only weak or no scientific proof. Gradually, however, research is starting to support this change, as more prospective, randomised trials comparing BPTB and hamstring tendon autografts have been published during the last decade (21, 23, 26). The hamstring tendon graft appears to have advantages compared with the BPTB autograft, mainly in terms of less donor-site morbidity. This opinion is further supported by the reported capacity of the ST tendon to regenerate (24, 79). Evaluating the hamstring tendons as thoroughly as the patellar tendon was evaluated in the present study appears to be an interesting perspective for future research.

Refining the present techniques might produce further improvement potential in ACL reconstructive surgery. Can stimulating growth factors improve the healing of the graft to its fixation sites? Is there an advantage in using hamstring autografts from the contralateral side? Is the so-called delayed reconstruction the optimal timing for surgery (91)? What is the ideal graft of the future? Is it one of the autografts already in use, an allograft or could it even be a cultured ligament? Will the synthetic ligament return? Could a direct suture of the injured ligament augmented with periosteum or some kind of allograft have a second chance? What about the results after ACL reconstruction using the double-tunnel technique? Involving the histological and ultrastructural aspects in all these questions could create ideas for numerous future projects.

En analys av det kliniska, radiologiska, histologiska och ultrastrukturella resultatet efter främre korsbandsrekonstruktion med autograft

Michael Svensson
Göteborg 2008

Abstrakt

Syftet med studierna var att utvärdera patellarsenan upp till sex år efter att dess centrala tredjedel skördats som graft vid främre korsbandskirurgi. Patellarsenan utvärderades upprepade gånger morfologiskt och histologiskt upptill sex år, samt ultrastrukturellt vid sex år efter skördning. Fynden jämfördes med frisk kontrollsena. Vidare jämfördes resultatet efter användning av patellarsenegraft (BPTB) och hamstringsenegraft (ST/G), avseende tagställesmorbiditet samt laxitet, efter främre korsbandskirurgi hos kvinnor.

Samtliga patienter rehabiliterades enligt standardiserade riktlinjer där full rörelseträning och belastning tilläts direkt postoperativt.

I en prospektiv studie analyserades tagstället efter främre korsbandskirurgi där den centrala tredjedelen av patellarsenan använts som graft (n=19). Upprepade magnetkameraundersökningar vid 6 veckor, 6 månader, 2 år samt 6 år postoperativt utfördes. Studien visade att patellarsenan inte normaliserades morfologiskt upptill sex år postoperativt jämfört med den friska icke-opererade sidan.

I en studie analyserades biopsier från patellarsenans centrala och laterala delar i elektron-mikroskop sex år postoperativt (n=13). En kvarstående oregelbundenhet återfanns i fiberstrukturen i patellarsenans centrala och laterala delar. Dessutom påvisades en signifikant förskjutning mot mindre diametrar av kollagen fibrillerna såväl centralt som lateralt i patellarsenan.

I en prospektiv studie analyserades patellarsenan histologiskt vid två respektive sex år postoperativt (n=17). Biopsier från patellarsenans centrala och laterala delar undersöktes i ljusmikroskop. Resultatet visade kvarstående histologiska förändringar med ökad celltäthet, ökad kärlförekomst samt kvarstående oregelbundenhet i patellarsenans fiberstruktur. Dessa förändringar var mest uttalade i patellarsenans centrala del, men noterades också i patellarsenans laterala del upp till sex år postoperativt.

I en studie jämfördes det postoperativa resultatet avseende tagställesmorbiditet och knälaxitet hos kvinnor efter främre korsbandsrekonstruktion utfört med antingen BPTB eller ST/G graft (n=63). Ingen signifikant skillnad avseende funktionella tester eller knälaxitet kunde påvisas mellan grupperna, men en signifikant reduktion i tagställesmorbiditet till fördel för ST/G gruppen påvisades två år postoperativt.

Sammanfattningsvis var studiernas viktigaste fynd att patellarsenan ej återfick normal morfologi, histologi eller ultrastruktur åtminstone upptill 6 år efter skördning av dess centrala tredjedel vid främre korsbandskirurgi. Dessa förändringar noterades inte enbart i den centrala delen av patellarsenan, varifrån graften skördats, utan också i den perifera tredjedelen av patellarsenan.

Acknowledgements

I would like to express my sincere gratitude to everyone who has made this thesis possible.

Jüri Kartus, Associate Professor at the Department of Orthopaedics at Norra Älvsborg/Uddevalla Hospital, my main tutor, colleague and friend. Thank you for pushing this project forward in good times and bad times alike. Your scientific sharpness and never-ending energy made this possible. I make no comment on your humour!

Jón Karlsson, Professor and Head of the Department of Orthopaedics, Sahlgrenska Academy. Thank you for your ability always to give support and take the time to talk not only about scientific matters.

Lars Karlsson, MD, Head of the Department of Orthopaedics at Norra Älvsborg/Uddevalla Hospital for giving me time and financial support to perform this work in spite of budget cuts.

Lars Ejerhed, MD, PhD at the Department of Orthopaedics at Norra Älvsborg/Uddevalla Hospital, for your constructive criticism and incisive comments.

Ninni Sernert, RPT, PhD at the Department of Orthopaedics at Norra Älvsborg/Uddevalla Hospital. Thank you for all your help pushing this project forward, without you this would have been practically impossible for me to fulfil.

Anna-Lena Wennerberg and Zarah Rosen, nurses at the Department of Orthopaedics at Norra Älvsborg/Uddevalla Hospital, for your commitment in finding and calling patients to every investigation.

Lisbeth Andersson, secretary at the Department of Orthopaedics at Norra Älvsborg/Uddevalla Hospital, for helping me with all the paperwork and practical matters.

Janne Rak, hospital photographer at Norra Älvsborg/Uddevalla Hospital, for excellent photos and posters, and always managing to be ready in time.

Catarina Kartus, for your excellent illustrations in the thesis.

Jeanette Kliger, for your excellent linguistic revision.

Linda Johansson, for assistance in the practical matter of preparing the dissertation

and for your unparalleled knowledge of the pathways within the university.

Lars Rostgård-Christensen, MD at the Department of Radiology at Norra Älvsborg/Uddevalla Hospital, for performing all the biopsy procedures.

Sven Lindahl, MD, PhD previously at the Department of Radiology at Norra Älvsborg/Uddevalla Hospital, for reading all the MRI examinations.

Tomas Movin, MD, PhD at the Department of Orthopaedics, Karolinska University Hospital/Huddinge, Karolinska Institutet, Stockholm, for examining all the histological samples together with **Nikos Papadogiannakis,** PhD, at the Department of Pathology, Karolinska University Hospital/Huddinge, Karolinska Institutet, Stockholm.

Kjell Hultenby, PhD at the Department of Pathology, Karolinska University Hospital/Huddinge, Karolinska Institutet, Stockholm, for assessing all the ultrastructural evaluations together with **Eva Blomén,** PhD at the Department of Pathology, Karolinska University Hospital/Huddinge, Karolinska Institutet, Stockholm.

Erling Hallström, MD at the Department of Orthopaedics at Norra Älvsborg/Uddevalla Hospital, colleague and friend, for always finding time for a chat and always being ready to laugh

Colleagues, at the Department of Orthopaedics at Norra Älvsborg/Uddevalla Hospital for supporting me, especially when it came to personal matters. For being good friends at all times and for making it possible for me to take time off work to do research.

All my friends, for your support for me and my family at all times.

My family, Yvonne, Alexander and Alicia, for supporting this project, even if it stole time from you in times of sickness and pain. I hope that our future looks brighter. To my parents, who left this life much too early.

This thesis has been supported by grants from the Swedish Centre for Research in Sports, the Research and Development Department at Norra Älvsborg/ Uddevalla Hospital and the County Council of Western Sweden.

References

1. Adam F, Pape D, Kohn D, Seil R. Length of the patellar tendon after anterior cruciate ligament reconstruction with patellar tendon autograft: a prospective clinical study using Roentgen stereometric analysis. Arthroscopy 2002;18:859-864.
2. Aglietti P, Buzzi R, Zaccherotti G, De Biase P. Patellar tendon versus doubled semitendinosus and gracilis tendons for anterior cruciate ligament reconstruction. Am J Sports Med 1994;22:211-217.
3. Amiel D, Frank C, Harwood F, Fronek J, Akeson W. Tendons and ligaments: a morphological and biochemical comparison. J Orthop Res 1984;1:257-265.
4. Arendt E, Dick R. Knee injury patterns among men and women in collegiate basketball and soccer. NCAA data and review of literature. Am J Sports Med 1995;23:694-701.
5. Aune AK, Holm I, Risberg MA, Jensen HK, Steen H. Four-strand hamstring tendon autograft compared with patellar tendon- bone autograft for anterior cruciate ligament reconstruction. A randomized study with two-year follow-up. Am J Sports Med 2001;29:722-728.
6. Barber-Westin SD, Noyes FR, Andrews M. A rigorous comparison between the sexes of results and complications after anterior cruciate ligament reconstruction. Am J Sports Med 1997;25:514-526.
7. Barber SD, Noyes FR, Mangine RE, McCloskey JW, Hartman W. Quantitative assessment of functional limitations in normal and anterior cruciate ligament-deficient knees. Clin Orthop 1990;255:204-214.
8. Barrett GR, Noojin FK, Hartzog CW, Nash CR. Reconstruction of the anterior cruciate ligament in females: A comparison of hamstring versus patellar tendon autograft. Arthroscopy 2002;18:46-54.
9. Battlehner CN, Carneiro Filho M, Ferreira Junior JM, Saldiva PH, Montes GS. Histochemical and ultrastructural study of the extracellular matrix fibers in patellar tendon donor site scars and normal controls. J Submicrosc Cytol Pathol 1996;28:175-186.
10. Berg EE. Intrinsic healing of a patellar tendon donor site defect after anterior cruciate ligament reconstruction. Clin Orthop 1992;278:160-163.
11. Bernicker JP, Haddad JL, Lintner DM, DiLiberti TC, Bocell JR. Patellar tendon defect during the first year after anterior cruciate ligament reconstruction: appearance on serial magnetic resonance imaging. Arthroscopy 1998;14:804-809.
12. Bjordal JM, Arnly F, Hannestad B, Strand T. Epidemiology of anterior cruciate ligament injuries in soccer. Am J Sports Med 1997;25:341-345.
13. Brandsson S, Faxén E, Kartus J, Jerre R, Eriksson BI, Karlsson J. A prospective four- to seven-year follow-up after arthroscopic anterior cruciate ligament reconstruction. Scand J Med Sci Sports 2001;11:23-27.
14. Brosseau L, Balmer S, Tousignant M, O'Sullivan JP, Goudreault C, Goudreault M, Gringras S. Intra- and intertester reliability and criterion validity of the parallelogram and universal goniometers for measuring maximum active knee flexion and extension of patients with knee restrictions. Arch Phys Med Rehabil 2001;82:396-402.

15. Burks RT, Haut RC, Lancaster RL. Biomechanical and histological observations of the dog patellar tendon after removal of its central one-third. Am J Sports Med 1990;18:146-153.
16. Buss DD, Warren RF, Wickiewicz TL, Galinat BJ, Panariello R. Arthroscopically assisted reconstruction of the anterior cruciate ligament with use of autogenous patellar-ligament grafts. Results after twenty-four to forty-two months. J Bone Joint Surg [Am] 1993;75:1346-1355.
17. Corry IS, Webb JM, Clingeleffer AJ, Pinczewski LA. Arthroscopic reconstruction of the anterior cruciate ligament. A comparison of patellar tendon autograft and four-strand hamstring tendon autograft. Am J Sports Med 1999;27:444-454.
18. Coupens SD, Yates CK, Sheldon C, Ward C. Magnetic resonance imaging evaluation of the patellar tendon after use of its central one-third for anterior cruciate ligament reconstruction. Am J Sports Med 1992;20:332-335.
19. Daniel DM. Reference, Maintenance & User´s Guide for the Knee Ligament Arthrometer. MEDmetric Corporation, 7542 Trade Street, San Diego, California 1993.
20. Daniel M, Malcolm L, Stone M, Perth H, Morgan J, Riehl B. Quantification of knee stability and function. Contemp Orthop 1982;5:83-91.
21. Ejerhed L, Kartus J, Sernert N, Kohler K, Karlsson J. Patellar Tendon or Semitendinosus Tendon Autografts for Anterior Cruciate Ligament Reconstruction?: A Prospective Randomized Study with a Two-Year Follow-up. Am J Sports Med 2003;31:19-25.
22. Engstrom B, Johansson C, Tornkvist H. Soccer injuries among elite female players. Am J Sports Med 1991;19:372-375.
23. Eriksson K, Anderberg P, Hamberg P, Lofgren AC, Bredenberg M, Westman I, Wredmark T. A comparison of quadruple semitendinosus and patellar tendon grafts in reconstruction of the anterior cruciate ligament. J Bone Joint Surg [Br] 2001;83:348-354.
24. Eriksson K, Larsson H, Wredmark T, Hamberg P. Semitendinosus tendon regeneration after harvesting for ACL reconstruction. A prospective MRI study. Knee Surg Sports Traumatol Arthrosc 1999;7:220-225.
25. Feagin JA, Jr., Wills RP, Lambert KL, Mott HW, Cunningham RR. Anterior cruciate ligament reconstruction. Bone-patella tendon-bone versus semitendinosus anatomic reconstruction. Clin Orthop Relat Res 1997;341:69-72.
26. Feller JA, Webster KE. A randomized comparison of patellar tendon and hamstring tendon anterior cruciate ligament reconstruction. Am J Sports Med 2003;31:564-573.
27. Ferrari JD, Bach BR, Jr., Bush-Joseph CA, Wang T, Bojchuk J. Anterior cruciate ligament reconstruction in men and women: An outcome analysis comparing gender. Arthroscopy 2001;17:588-596.
28. Ferretti A, Papandrea P, Conteduca F, Mariani PP. Knee ligament injuries in volleyball players. Am J Sports Med 1992;20:203-207.
29. Fiebert I, Gresley J, Hoffman S, Kunkel K. Comparative measurements of anterior tibial translation using the KT-1000 knee arthrometer with the leg in neutral,

internal rotation, and external rotation. J Orthop Sports Phys Ther 1994;19:331-334.
30. Freedman KB, D'Amato MJ, Nedeff DD, Kaz A, Bach BR, Jr. Arthroscopic anterior cruciate ligament reconstruction: a metaanalysis comparing patellar tendon and hamstring tendon autografts. Am J Sports Med 2003;31:2-11.
31. Fu FH, Bennett CH, Ma CB, Menetrey J, Lattermann C. Current trends in anterior cruciate ligament reconstruction. Part II. Operative procedures and clinical correlations. Am J Sports Med 2000;28:124-130.
32. Gobbi A, Mahajan S, Zanazzo M, Tuy B. Patellar tendon versus quadrupled bone-semitendinosus anterior cruciate ligament reconstruction: a prospective clinical investigation in athletes. Arthroscopy 2003;19:592-601.
33. Gray J, Taunton JE, McKenzie DC, Clement DB, McConkey JP, Davidson RG. A survey of injuries to the anterior cruciate ligament of the knee in female basketball players. Int J Sports Med 1985;6:314-316.
34. Gustavsson A, Neeter C, Thomee P, Gravare Silbernagel K, Augustsson J, Thomee R, Karlsson J. A test battery for evaluating hop performance in patients with an ACL injury and patients who have undergone ACL reconstruction. Knee Surg Sports Traumatol Arthrosc 2006;14:778-788.
35. Gwinn DE, Wilckens JH, McDevitt ER, Ross G, Kao TC. The relative incidence of anterior cruciate ligament injury in men and women at the United States Naval Academy. Am J Sports Med 2000;28:98-102.
36. Harmon KG, Ireland ML. Gender differences in noncontact anterior cruciate ligament injuries. Clin Sports Med 2000;19:287-302.
37. Harner CD, Irrgang JJ, Paul J, Dearwater S, Fu FH. Loss of motion after anterior cruciate ligament reconstruction. Am J Sports Med 1992;20:499-506.
38. Hefti F, Müller W, Jakob RP, Stäubli HU. Evaluation of knee ligament injuries with the IKDC form. Knee Surg Sports Traumatol Arthrosc 1993;1:226-234.
39. Hiemstra LA, Webber S, MacDonald PB, Kriellaars DJ. Knee strength deficits after hamstring tendon and patellar tendon anterior cruciate ligament reconstruction. Med Sci Sports Exerc 2000;32:1472-1479.
40. Hirokawa S, Solomonow M, Lu Y, Lou ZP, D'Ambrosia R. Anterior-posterior and rotational displacement of the tibia elicited by quadriceps contraction. Am J Sports Med 1992;20:299-306.
41. Hoher J, Bach T, Münster A, Bouillon B, Tiling T. Does the mode of data collection change results in a subjective knee score? Self-administration versus interview. Am J Sports Med 1997;25:642-647.
42. Huber FE, Irrgang JJ, Harner C, Lephart S. Intratester and intertester reliability of the KT-1000 arthrometer in the assessment of posterior laxity of the knee. Am J Sports Med 1997;25:479-485.
43. Huston LJ, Wojtys EM. Neuromuscular performance characteristics in elite female athletes. Am J Sports Med 1996;24:427-436.
44. Irrgang JJ, Harner CD. Loss of motion following knee ligament reconstruction. Sports Med 1995;19:150-159.
45. Johnson DL. Graft Choise for ACL Reconstruction 2006; www.isakos.com, current concepts.

.

46. Jozsa L, Balint BJ, Reffy A, Demel Z. Fine structural alterations of collagen fibers in degenerative tendinopathy. Arch Orthop Trauma Surg 1984;103:47-51.
47. Kartus J, Ejerhed L, Eriksson BI, Karlsson J. The localization of the infrapatellar nerves in the anterior knee region with special emphasis on central third patellar tendon harvest: a dissection study on cadaver and amputated specimens. Arthroscopy 1999;15:577-586.
48. Kartus J, Ejerhed L, Sernert N, Brandsson S, Karlsson J. Comparison of traditional and subcutaneous patellar tendon harvest. A prospective study of donor site-related problems after anterior cruciate ligament reconstruction using different graft harvesting techniques. Am J Sports Med 2000;28:328-335.
49. Kartus J, Lindahl S, Kohler K, Sernert N, Eriksson BI, Karlsson J. Serial magnetic resonance imaging of the donor site after harvesting the central third of the patellar tendon. A prospective study of 37 patients after arthroscopic anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 1999;7:20-24.
50. Kartus J, Magnusson L, Stener S, Brandsson S, Eriksson BI, Karlsson J. Complications following arthroscopic anterior cruciate ligament reconstruction. A 2-5-year follow-up of 604 patients with special emphasis on anterior knee pain. Knee Surg Sports Traumatol Arthrosc 1999;7:2-8.
51. Kartus J, Movin T, Karlsson J. Donor-site morbidity and anterior knee problems after anterior cruciate ligament reconstruction using autografts. Arthroscopy 2001;17:971-980.
52. Kartus J, Movin T, Papadogiannakis N, Christensen LR, Lindahl S, Karlsson J. A radiographic and histologic evaluation of the patellar tendon after harvesting its central third. Am J Sports Med 2000;28:218-226.
53. Kartus J, Stener S, Lindahl S, Engstrom B, Eriksson BI, Karlsson J. Factors affecting donor-site morbidity after anterior cruciate ligament reconstruction using bone-patellar tendon-bone autografts. Knee Surg Sports Traumatol Arthrosc 1997;5:222-228.
54. Kartus J, Stener S, Lindahl S, Eriksson BI, Karlsson J. Ipsi- or contralateral patellar tendon graft in anterior cruciate ligament revision surgery. A comparison of two methods. Am J Sports Med 1998;26:499-504.
55. Kim DH, Wilson DR, Hecker AT, Jung TM, Brown CH, Jr. Twisting and braiding reduces the tensile strength and stiffness of human hamstring tendon grafts used for anterior cruciate ligament reconstruction. Am J Sports Med 2003;31:861-867.
56. Kurosaka M, Yoshiya S, Andrish JT. A biomechanical comparison of different surgical techniques of graft fixation in anterior cruciate ligament reconstruction. Am J Sports Med 1987;15:225-229.
57. Kvist J, Gillquist J. Sagittal plane knee translation and electromyographic activity during closed and open kinetic chain exercises in anterior cruciate ligament-deficient patients and control subjects. Am J Sports Med 2001;29:72-82.
58. LaPrade RF, Hamilton CD, Montgomery RD, Wentorf F, Hawkins HD. The reharvested central third of the patellar tendon. A histologic and biomechanical analysis. Am J Sports Med 1997;25:779-785.

59. Lavagnino M, Arnoczky SP, Egerbacher M, Gardner KL, Burns ME. Isolated fibrillar damage in tendons stimulates local collagenase mRNA expression and protein synthesis. J Biomech 2006;39:2355-2362.
60. Laxdal G, Kartus J, Ejerhed L, Sernert N, Magnusson L, Faxén E, Karlsson J. Outcome and risk factors after anterior cruciate ligament reconstruction: a follow-up study of 948 patients. Arthroscopy 2005;21:958-964.
61. Laxdal G, Kartus J, Hansson L, Heidvall M, Ejerhed L, Karlsson J. A prospective randomized comparison of bone-patellar tendon-bone and hamstring grafts for anterior cruciate ligament reconstruction. Arthroscopy 2005;21:34-42.
62. Liu SH, Hang DW, Gentili A, Finerman GA. MRI and morphology of the insertion of the patellar tendon after graft harvesting. J Bone Joint Surg [Br] 1996;78:823-826.
63. Maffulli N, Thorpe AP, Smith EW. Magnetic resonance imaging after operative repair of achilles tendon rupture. Scand J Med Sci Sports 2001;11:156-162.
64. Magnusson SP, Qvortrup K, Larsen JO, Rosager S, Hanson P, Aagaard P, Krogsgaard M, Kjaer M. Collagen fibril size and crimp morphology in ruptured and intact Achilles tendons. Matrix Biol 2002;21:369-377.
65. Majima T, Yasuda K, Tsuchida T, Tanaka K, Miyakawa K, Minami A, Hayashi K. Stress shielding of patellar tendon: effect on small-diameter collagen fibrils in a rabbit model. J Orthop Sci 2003;8:836-841.
66. Malcom LL, Daniel DM, Stone ML, Sachs R. The measurement of anterior knee laxity after ACL reconstructive surgery. Clin Orthop 1985;196:35-41.
67. Marder RA, Raskind JR, Carroll M. Prospective evaluation of arthroscopically assisted anterior cruciate ligament reconstruction. Patellar tendon versus semitendinosus and gracilis tendons. Am J Sports Med 1991;19:478-484.
68. Meisterling RC, Wadsworth T, Ardill R, Griffiths H, Lane-Larsen CL. Morphologic changes in the human patellar tendon after bone-tendon-bone anterior cruciate ligament reconstruction. Clin Orthop 1993;289:208-212.
69. Moller M, Kalebo P, Tidebrant G, Movin T, Karlsson J. The ultrasonographic appearance of the ruptured Achilles tendon during healing: a longitudinal evaluation of surgical and nonsurgical treatment, with comparisons to MRI appearance. Knee Surg Sports Traumatol Arthrosc 2002;10:49-56.
70. Movin T, Gad A, Reinholt FP, Rolf C. Tendon pathology in long-standing achillodynia. Biopsy findings in 40 patients. Acta Orthop Scand 1997;68:170-175.
71. Muellner T, Kwasny O, Loehnert V, Mallinger R, Unfried G, Schabus R, Plenk H, Jr. Light and electron microscopic study of stress-shielding effects on rat patellar tendon. Arch Orthop Trauma Surg 2001;121:561-565.
72. Muneta T, Ezura Y, Sekiya I, Yamamoto H. Anterior knee laxity and loss of extension after anterior cruciate ligament injury. Am J Sports Med 1996;24:603-607.
73. Myklebust G, Maehlum S, Holm I, Bahr R. A prospective cohort study of anterior cruciate ligament injuries in elite Norwegian team handball. Scand J Med Sci Sports 1998;8:149-153.
74. Nakamura N, Horibe S, Sasaki S, Kitaguchi T, Tagami M, Mitsuoka T, Toritsuka Y, Hamada M, Shino K. Evaluation of active knee flexion and hamstring strength

after anterior cruciate ligament reconstruction using hamstring tendons. Arthroscopy 2002;18:598-602.
75. Nixon RG, SeGall GK, Sax SL, Cain TE, Tullos HS. Reconstitution of the patellar tendon donor site after graft harvest. Clin Orthop 1995;317:162-171.
76. Noojin FK, Barrett GR, Hartzog CW, Nash CR. Clinical comparison of intraarticular anterior cruciate ligament reconstruction using autogenous semitendinosus and gracilis tendons in men versus women. Am J Sports Med 2000;28:783-789.
77. Ostenberg A, Roos H. Injury risk factors in female European football. A prospective study of 123 players during one season. Scand J Med Sci Sports 2000;10:279-285.
78. Paessler HH, Mastrokalos DS. Anterior cruciate ligament reconstruction using semitendinosus and gracilis tendons, bone patellar tendon, or quadriceps tendon-graft with press-fit fixation without hardware. A new and innovative procedure. Orthop Clin North Am 2003;34:49-64.
79. Papandrea P, Vulpiani MC, Ferretti A, Conteduca F. Regeneration of the semitendinosus tendon harvested for anterior cruciate ligament reconstruction. Evaluation using ultrasonography. Am J Sports Med 2000;28:556-561.
80. Petschnig R, Baron R, Albrecht M. The relationship between isokinetic quadriceps strength test and hop tests for distance and one-legged vertical jump test following anterior cruciate ligament reconstruction. J Orthop Sports Phys Ther 1998;28:23-31.
81. Pinczewski LA, Deehan DJ, Salmon LJ, Russell VJ, Clingeleffer A. A five-year comparison of patellar tendon versus four-strand hamstring tendon autograft for arthroscopic reconstruction of the anterior cruciate ligament. Am J Sports Med 2002;30:523-536.
82. Powell JW, Barber-Foss KD. Sex-related injury patterns among selected high school sports. Am J Sports Med 2000;28:385-391.
83. Proctor CS, Jackson DW, Simon TM. Characterization of the repair tissue after removal of the central one- third of the patellar ligament. An experimental study in a goat model. J Bone Joint Surg [Am] 1997;79:997-1006.
84. Roos H, Ornell M, Gardsell P, Lohmander LS, Lindstrand A. Soccer after anterior cruciate ligament injury--an incompatible combination? A national survey of incidence and risk factors and a 7-year follow-up of 310 players. Acta Orthop Scand 1995;66:107-112.
85. Rosene J, TD F. Anterior tibial translation in collegiate athletes with normal anterior cruciate ligament integrity. J Athletic Training 1999;34:93-98.
86. Rozzi SL, Lephart SM, Gear WS, Fu FH. Knee joint laxity and neuromuscular characteristics of male and female soccer and basketball players. Am J Sports Med 1999;27:312-319.
87. Sanchis-Alfonso V, Subias-Lopez A, Monteagudo-Castro C, Rosello-Sastre E. Healing of the patellar tendon donor defect created after central-third patellar tendon autograft harvest. A long-term histological evaluation in the lamb model. Knee Surg Sports Traumatol Arthrosc 1999;7:340-348.

88. Sernert N, Kartus J, Kohler K, Ejerhed L, Brandsson S, Karlsson J. Comparison of functional outcome after anterior cruciate ligament reconstruction resulting in low, normal and increased laxity. Scand J Med Sci Sports 2002;12:47-53.
89. Shaieb MD, Kan DM, Chang SK, Marumoto JM, Richardson AB. A prospective randomized comparison of patellar tendon versus semitendinosus and gracilis tendon autografts for anterior cruciate ligament reconstruction. Am J Sports Med 2002;30:214-220.
90. Shelbourne KD, Nitz P. Accelerated rehabilitation after anterior cruciate ligament reconstruction. Am J Sports Med 1990;18:292-299.
91. Shelbourne KD, Patel DV, Martini DJ. Classification and management of arthrofibrosis of the knee after anterior cruciate ligament reconstruction. Am J Sports Med 1996;24:857-862.
92. Smith RK, Zunino L, Webbon PM, Heinegard D. The distribution of cartilage oligomeric matrix protein (COMP) in tendon and its variation with tendon site, age and load. Matrix Biol 1997;16:255-271.
93. Soderman K, Alfredson H, Pietila T, Werner S. Risk factors for leg injuries in female soccer players: a prospective investigation during one out-door season. Knee Surg Sports Traumatol Arthrosc 2001;9:313-321.
94. Strand T, Solheim E. Clinical tests versus KT-1000 instrumented laxity test in acute anterior cruciate ligament tears. Int J Sports Med 1995;16:51-53.
95. Tegner Y, Lysholm J. Rating systems in the evaluation of knee ligament injuries. Clin Orthop 1985;198:43-49.
96. Tegner Y, Lysholm J, Lysholm M, Gillquist J. A performance test to monitor rehabilitation and evaluate anterior cruciate ligament injuries. Am J Sports Med 1986;14:156-159.
97. Torg JS, Conrad W, Kalen V. Clinical diagnosis of anterior cruciate ligament instability in the athlete. Am J Sports Med 1976;4:84-93.
98. Tuite DJ, Renstrom PA, O'Brien M. The aging tendon. Scand J Med Sci Sports 1997;7:72-77.
99. Wiger P, Brandsson S, Kartus J, Eriksson BI, Karlsson J. A comparison of results after arthroscopic anterior cruciate ligament reconstruction in female and male competitive athletes. A two- to five- year follow-up of 429 patients. Scand J Med Sci Sports 1999;9:290-295.
100. Wiggins DL, Wiggins ME. The female athlete. Clin Sports Med 1997;16:593-612.
101. Wikstrom B, Hjerpe A, Hultenby K, Reinholt FP, Engfeldt B. Stereological analysis of the epiphyseal growth cartilage in the brachymorphic (bm/bm) mouse, with special reference to the distribution of matrix vesicles. Virchows Arch B Cell Pathol Incl Mol Pathol 1984;47:199-210.
102. Wiley JP, Bray RC, Wiseman DA, Elliott PD, Ladly KO, Vale LA. Serial ultrasonographic imaging evaluation of the patellar tendon after harvesting its central one third for anterior cruciate ligament reconstruction. J Ultrasound Med 1997;16:251-255.
103. Yeung EW, Yeung SS. A systematic review of interventions to prevent lower

limb soft tissue running injuries. Br J Sports Med 2001;35:383-389.
104. Yunes M, Richmond JC, Engels EA, Pinczewski LA. Patellar versus hamstring tendons in anterior cruciate ligament reconstruction: A meta-analysis. Arthroscopy 2001;17:248-257.

Studies I-IV